

# nature

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## Royal Society Esso Award

Rp385

## for the Conservation of Energy

The Royal Society Esso Award for 1975 has been made jointly and equally to Professor H. C. Hottel of the Massachusetts Institute of Technology and Professor H. Tabor of the Israel Scientific Research Foundation. The Award has been made to Professor Hottel for work on problems of solar energy utilisation, particularly in solar-heated houses. Professor Tabor received the Award for his work on the theory of selective surfaces, also on increasing efficiency of solar energy collectors, including the design of focusing collectors, and the development of turbines for operation on solar energy.

The Royal Society Esso Award for the Conservation of Energy was instituted in 1974 and consists of a gold medal and a prize of £1,000 offered for outstanding contributions to the advance of science, engineering or technology, leading to the more efficient conversion or use of any form of energy. **Nominations for the 1976 Award are required by 2 February 1976.** For further details, please contact  
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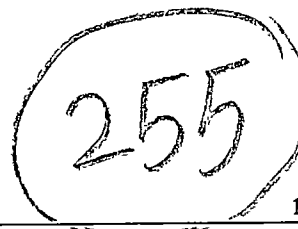
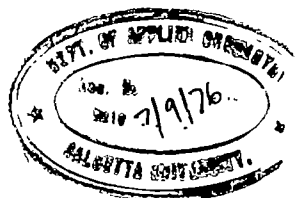
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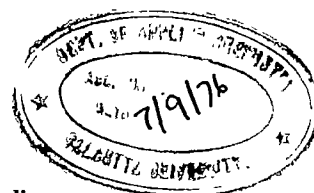
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Cover picture

The pumice cliffs of Santorini  
(see page 194)

Photo: J. Allan Cash



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The position of *Tarsus* in the evolu-  
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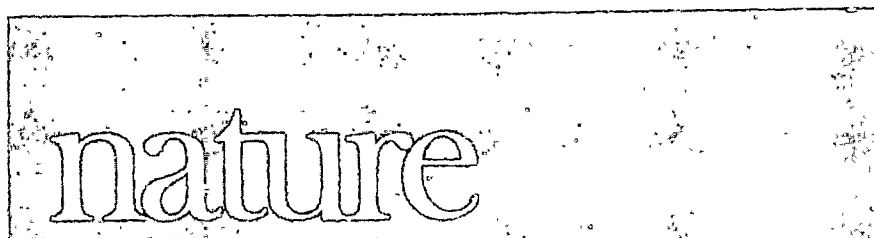
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Libya—the immense sand dunes of  
southern Fezzan. See page 653.

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● Articles are up to 3,000 words in length with at most six displayed items (figures and tables) and may either be reports of major research developments in a subject or broader reviews of progress.

● Letters are brief reports of research of unusual and wide interest, not in general longer than 1,000 words; at most they have three or four displayed items (figures and tables).

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**nature**

January 1 &amp; 8, 1976

## Snooping with a CRT

A nurse with access to health records by means of a computer terminal types in her boy-friend's name and finds his medical history. It is not all she would have hoped for. She breaks it off with him.

A defendant in a well publicised civil case has an alibi which the court accepts. A credit card company employee, out of curiosity, calls up his file and finds that his alibi is not borne out by the list of his transactions.

A hotel booking clerk uses a computer terminal to check on a putative guest and finds that a year ago, in a different hotel, he left without paying for his room. The guest, immediately on discovering his oversight, had paid the bill by post. But this correction never got into the computer. He is told the hotel is full.

THESE are three of the diverse ways in which computers can facilitate information-collecting in somewhat murky circumstances. The Home Office has recently published a White Paper on Computers and Privacy (Cmd 6353, 28p), and with it a summary of an interdepartmental working party's review of computer usage in the public sector, with particular reference to safeguards to protect privacy and confidentiality. Back in 1972 Sir Kenneth Younger's Committee on Privacy reported that, although there was no evidence that the private sector was using computers to threaten privacy, there was need for vigilance, in the form of an independent body to review the gathering and processing of personal information. The Younger Committee also gave the government gratuitous advice that such an independent body could, in addition, study the public sector. This White Paper advances the cause of a Data Protection Authority to oversee the handling of personal information. It also gives some reassurance that, in as far as the evidence exists, the use of computers to impinge on people's private lives is not on the increase.

Privacy is a misnomer. We are really talking of the use of personal information for purposes other than that for which it was supplied or collected, and the use of inaccurate or incomplete data where the person reported on has no chance to challenge the accuracy or completeness.

Computers is also a misnomer, so to say. Computers have not, up to the present, created new opportunities for misuse of information; they have simply made

possible quicker and larger operations, allowing instant decisions to be made. Newspapers, or gossip in the staff canteen, still have the potential for much more damaging intrusions into the life of the individual than do computers. Nonetheless the computer, with its 1984 image, attracts a lot of the fire, and is often credited with monitoring skills that it does not possess.

The average reader of *Nature* would, however, probably settle for more automation in dealing with public and private enterprise. The tedium of paying separate bills for mortgage, licences, utilities, insurance—of having different numbers for health services, tax authorities, passport, bank, telephone, credit cards—often niggles; roll on rationalisation. But one third of the population of the UK regards it as an invasion of privacy even to have publicly available lists of names and addresses such as electoral lists or telephone directories. With such extremes of opinion, is there any hope that a statutory agency such as the White Paper envisages can satisfy everyone that information is not being misused?

The Younger Committee put forward ten principles for the handling of personal information in computers, including one that "there should be arrangements whereby the subject could be told about the information held concerning him". The White Paper proposes more—"the subject should also be able to find out what has been done with the information, and to whom it has been given". In a society of technocrats, we would all receive a weekly listing of what was held in data banks about us, and by whom that information had been used; the big advantage of storing information in computers, of course, is that all usage can be logged. If we found someone had been nosing around without authority, we would call our lawyer. But that one third of the nation which does not even want names and addresses listed probably has little idea of what to do if supplied with computer print-outs. It certainly is not in the habit of consulting lawyers.

Here, then, is a real job for the Data Protection Authority—to make sure not only that everyone knows what the information they have provided is being used for, but also that ways are devised for fighting misuse at the level of the individual. Many of the biggest threats to the individual, after all, do not come from the computer at all. But perhaps an imaginatively appointed Data Protection Authority, not stuffed with "the good and the great", might lead the way towards broader initiatives to look after those in greatest danger of being trampled on.



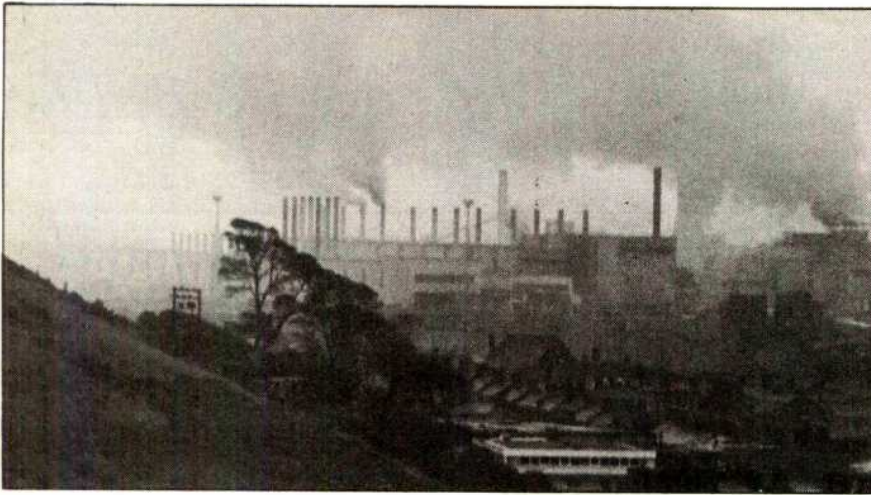


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## Can Europe clean itself up?

*Despite the rhetoric that bursts forth when principles of European Economic Community action are outlined, environment policy remains something of a backward region. Progress towards common action at Community level has been tardy, not through a lack of ideas but a paucity of decisions. Paul Cheeseright reports.*

IN THE EEC, environment is a policy looking for an executor. Yet opinion polls would suggest that a more vigorous approach by the Community's decision-making arm, the Council of Ministers, would not be lacking in some sort of popular acclaim. Nearly 10,000 people questioned throughout the Community in the middle of last year were asked to list the most important problems facing the Community. Second on the list of five major concerns came nature conservancy, and most of the interviewees favoured European action to deal with all of the problems.

In fact, a vast amount of work into the problems and techniques of environmental control is proceeding within the Community. The great problem, however, is that most of it is uncoordinated, and at Brussels there is simply inadequate information to gain a coherent picture. For this reason, it was agreed towards the end of last year that the European Commission, the ideas centre and civil service section of the Community, should pull together an inventory of sources of information on the environment. The data would be supplied by the nine member states and include independent research exercises. The information supplied would be processed in such a way as to be compatible with similar groundwork being undertaken for the UN international reference system on the environment.

This measure extends the interchange of information that was begun in 1973 when the Community took its first halting steps towards harmonising national legislation on the environ-

ment. When one state desires to take action in an area where the Commission has not presented proposals, the measure can only come into effect six months after the Commission has been informed and only if the Commission has presented no proposals to the Council. Under an agreement of March 1973, the Commission is in any case informed of draft laws, regulations or administrative provisions. In the first two years after the agreement it was informed by the member states of 22 laws or regulations, 67 draft provisions and six international agreements, the greater part coming from Denmark, France and Germany.

The existence of such mechanisms acts as a check on the use of environmental policy in a purely chauvinistic sense, and is an acceptance of the principle that environmental policy applied in any determined fashion is a nonsense when conceived in a purely national framework. Indeed, one of the lessons of the international conference at Stockholm in June 1972 was that in the absence of any international organisation to supervise the improvement of the European environment, only the Community can execute a policy that transcends national frontiers. Yet the Community has only limited powers, and over the next decade it seems likely that any steps taken will work towards the mitigation of problems rather than their cure or outright prevention. This is not to say that water will not become purer or the air cleaner, but it is to say that there are certain forces at work within Europe that will not be pushed aside.

Among them is the demographic trend which even before 1970 had shown that 30% of the population of France, Germany, Italy and the Benelux countries was concentrated in 9% of the available land area. Further, in the area of greatest population concentration, the birth rate was found to be more than double that of the outlying regions. The result of this trend could be to entrench the so-called Golden Triangle, the industrial area that extends from the Midlands of England to the Gulf of Genoa. Any environmental policy is inevitably linked to balanced development. But this is not taking place, and, indeed, the Community's agricultural policy is actually encouraging people off the land into the industrial conurbations.

Under the liberal capitalist economic systems of the Nine, new industries are usually attracted to the main market areas, thus strengthening the tendencies towards pollution that have been created by a century's unplanned search for industrial prosperity. In the strictest terms, then, a successful Community environment policy demands some control over the disposition of economic activity. But there is no power to create such a system of control, nor much evidence of any desire to create it. It would involve the emergence of a supranational state which, while it might be the aim of the European integrationists, is scarcely a realistic political concept for the time being. Community environment policy is likely to remain something of a patchwork while the politicians strive to come to terms with what the Community's Altiero Spinelli once called "a spectrum of complex interdisciplinary problems, whose impact, interconnection and consequences are frequently not completely known as yet." There is the additional problem that the environment as such is not mentioned in the Treaty of Rome and that, freed from the legal requirements of common action, the member states' movements on an integrated policy will be a direct reflection of their somewhat haphazard desire to move closer together politically.

It is against this background that the Community has been formulating and enacting a limited environment policy. The Community was a relative late-comer to the field. It was not until 1971 that the Commission established a special unit to deal with policy, and it was only at the Community summit meeting of October 1972 that their efforts received much political recognition. A detailed action programme was ordered for presentation in the middle of 1973. This programme was adopted later and ran until the end of 1975. Now a further programme to cover the next five years, with revision after



2½ years, is under consideration.

The main thrust of the Community's first programme covered three broad areas. In the first place the aim was to prevent or reduce pollution; the second aim was to improve the environment generally and then, finally, to push forward Community involvement in the framework of international action on the environment. Within these general areas, the Community adopted the principle that "the polluter pays". This may turn out to be something of a misnomer, for the real fact is that the consumer pays. Where factories or agencies take steps to minimise their pollution of the environment, it is inevitable that this will be reflected in the prices charged for the products created. In addition, governments are using taxpayers' money to aid companies introducing anti-pollutant measures. The Community has since the end of 1974 had a general rule as to the apportionment of costs and the role of the public sector in providing aids for anti-pollutant measures. But this measure is less concerned with safeguarding public money than preventing the companies of one country gaining a competitive advantage over those of another.

The Community has also adopted a set of rather obvious principles for the administration of environment policy. There are eight in all, aimed above all at the co-ordination of national programmes. The use of natural resources which damage the ecological balance should be avoided, environmental damage should be prevented rather than corrected afterwards, technical progress must include efforts to protect the environment, the polluter should pay, the environment will be taken into consideration at an early stage of technical planning, activities in one country should not damage another and there should be nothing to impede the operation of the common market. The principles are banal, but significant in the European context mainly because they had not been enunciated before.

At the Community level, numerous research programmes have been set moving and plans for a European Foundation for the Improvement of Living and Working Conditions have been laid so that the Foundation should start working early this year. The Council has also adopted a resolution on the desirable quality of surface waters required for drinking and a recommendation on the storage and treatment of waste oil, and has adopted the Paris Convention for the prevention of marine pollution from land-based sources. At the end of 1975, the Council agreed that the Commission should represent the Community in international negotiations aimed at providing a basis on which it will be

possible to keep the Mediterranean cleared of pollution emanating from the land. In this case, the Commission's mandate is an extension of the agreement that was reached at the Paris Convention, which covered the North-East Atlantic. These examples give a flavour of the breadth of the work that is being undertaken at the Community level, although it can be argued cogently that the effort is minimal when related to the scale of the problems to be solved and the power of the Community when acting in a united fashion to solve them.

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**6 Given a problem it is possible to suggest a scientific counteraction. But Community problems are not dealt with according to such simple terms 9**

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But this really points up the difficulties that the Community is facing. Given a problem it is possible to suggest a scientific counteraction. But Community problems are not dealt with according to such simple terms. In the first place, there might be, and often is, argument about the scientific response to the problems because the geographical nature of the Community is so varied. In the second place, any scientific response to a problem has to be consistent with the overriding economic aims of the Community. Of course, the Community is concerned with economic growth, but the difficulty goes deeper than that into the existence of the common market. It is a dominant principle that the common market should not be endangered, that there should be free traffic in goods and that no company or country should have an advantage over another, created by the Community rules.

Two incidents in recent months provide evidence of the dilemma created by these circumstances in the Community. One instance remains unsolved. The other has been widened to embrace a compromise that might embrace the environmental end, while taking into account the nationally-based scientific and economic means.

The first relates to the content of lead in petrol, a subject which the Community has broached but has never been able to follow through because of the opposing national attitudes and interests. At the beginning of the year the West German Government was due to introduce a new regime governing the extent of lead permitted. In introducing stricter measures in the interests of curbing air pollution, the Germans have pursued a policy that is further advanced than that of other Community states. On January 1, the Germans reduced the legal lead content in petrol to 0.15 grams per litre from

0.4 grams. Precisely because the German regulations will be stepping outside the commercially accepted norms, the Commission has received complaints from a group of German oil companies and a number of Dutch firms that, as they are unable to meet the 0.15 grams regulations, they are being forced out of the market. Therefore, the companies argue, the regulation is an infringement of the principle of the common market and free competition. The case remains unresolved.

The second instance concerns the emission of toxic waste into the aquatic environment. It was the subject of bitter dispute between Britain and the other eight members of the Community at a Council meeting in October. A compromise was reached in December. Broadly the eight members wanted discharge controls for pollutants like cadmium, mercury, organohalogenic, organophosphoric and organotitanic composites, and a list of others making up a so-called black list Britain, on the other hand, argued that there should be an observance of quality control objectives—in other words, there should be no control on discharge where it could be indicated that the discharge did not affect the immediate environment. The eight contended that unless there was a uniform emission standard there would be distortion of economic competition. The British rejoinder was that this was nonsense and that there should be recognition that geographic factors were different in the varied parts of the Community. Britain had short rivers, a long shoreline and not many factories on the rivers. The compromise finally agreed was that emission standards should be used but there could be exceptions where quality objectives may be used.

While the Community, it may be argued, lost a chance, because of British intransigence, to move forward into the area of positive controls, it came face to face with the fact that environmental policy will have to be flexible in the future. The conditions in the north of Scotland are not the same as those in Sicily. If fish are not given a chance to live in the Rhine, they are breeding more heavily in the Thames. But beyond this, the flexibility of the approach to aquatic pollution is probably more in keeping with the political realities of 1975-6.

In a general sense, the environmental lobby is not as strong as it was in 1972-3, except insofar as it can bring its power to bear on local issues of immediate importance. And even if it was, it would not necessarily make much difference. Environmental policy in the Community has less to do with science and more to do with politics and economics. □

# international news

Just four years ago, President Nixon signed into law a piece of legislation which has fundamentally changed government support of biomedical research in the United States. Called the National Cancer Act, the legislation was designed, in Mr Nixon's words, to launch "a great crusade against cancer". The crusade has always been controversial, causing deep divisions within the biomedical research community about how it should be managed. But it has recently come in for some fresh criticism which bears especially close watching. In fact, the indications are that a major political debate about the structure and scope of the cancer research programme is beginning to take shape.

Even before Mr Nixon put his signature to the National Cancer Act, the measure was controversial. The chief bone of contention in the early days was the justifiable resistance within the scientific community to the notion that cancer research could be managed like an Apollo-style effort to land men on the Moon. Though concerns over the NASA-like approach persist, frequently finding expression in attacks on the huge amount of money being spent on efforts to track down human cancer viruses, criticisms of the cancer research crusade have recently coalesced around two arguments, both of which have been raised in debates in Congress in the past few weeks. The first is the argument that rapid increases in funds for cancer research have siphoned money from other, equally deserving, research programmes. And the second is the contention that the cancer crusade has concentrated on an elusive search for cures and treatments at the expense of prevention—in short, environmental causes of cancer have been relatively neglected.

The first complaint is by no means novel. In fact it was raised three years ago when Mr Nixon cut back research budgets across the board but spared the politically sensitive cancer programme from the axe. The matter took on a new dimension last September, however, when a group of Senators, led by Gaylord Nelson of Wisconsin and Alan Cranston of California, proposed an amendment to a budget bill which would have pared down the budget for the National Cancer Institute and redistributed some of the savings to other institutes of the National Institutes of Health (NIH).

The amendment was soundly defeated, but the issue of research priorities got a good airing on the Senate floor.

Armed with a sheaf of statistics and with letters of support from former NIH directors and top government health officials, Nelson and Cranston provided details of how the budgets have grown for cancer research and, to a lesser extent heart research (which was aided by the National Heart, Lung and Blood Act of 1972), while budgets

## Cancer controversy recommences

by Colin Norman, Washington

for other NIH institutes have languished. Between 1970 and 1975, the budget for the National Cancer Institute (NCI) rose by 280%, from \$182 million to \$669 million, and the budget for the National Heart and Lung Institute (NHLI) increased from \$159 million to \$303 million, while spending on all the other 11 institutes rose by only about 20%, from \$750 to \$908 million. In constant dollar terms, NCI's budget rose by 186%, NHLI's increased by 52%, while the rest of NIH suffered a 13% drop in funds.

Although few Senators were willing to vote for a redistribution of funds to less politically favoured research programmes, the matter of biomedical research priorities is now under intense scrutiny by a top-level Presidential commission which is examining NIH's research policies. Established last year by a bill sponsored by Senator Edward Kennedy, the commission is due to issue a report by April 30, and its findings are likely to spark a fundamental re-examination of biomedical research policies and priorities.

The second major complaint raised recently about the cancer crusade is that the effort is devoting insufficient attention to finding ways to prevent, rather than to treat or cure, cancers. The impetus for such complaints is generated by two factors. The first is the growing realisation that the majority of human cancers may be linked to environmental factors, such as carcinogens encountered in the work place and in the general environment (including cigarette smoke). The second is the fact that in spite of massive expenditures on cancer research, survival rates for people suffering from

many of the leading cancer killers have improved little since the 1950s.

Thus, Russell Train, the head of the Environmental Protection Agency, pointed out in a recent speech that as many as 60–90% of human cancers can be traced to environmental causes, and he suggested that "we may be approaching the whole question of human health from the wrong side . . . an ounce of prevention may well be worth a pound of cure".

Similar sentiments were expressed more bluntly last year by a sub-committee of the National Cancer Advisory Board, NCI's top advisory body. The sub-committee, which was chaired by Dr Philippe Shubik of the University of Nebraska, expressed its "sense of general astonishment" that the cancer programme "does not appear to have accorded an adequate priority nor sense of urgency to the field of environmental carcinogenesis". The sub-committee's report went on to recommend that more money be put into chemical carcinogenesis (it estimated that less than 10% of NCI's budget is now spent on such studies) and it suggested that if necessary some money should be reprogrammed from other fields, such as tumour virology.

NCI officials have generally countered that they are spending much more than 10% of their budget on environmental carcinogenesis, and that if good research proposals in that field come along, they will certainly be funded. Nevertheless, complaints about lack of attention to environmental carcinogenesis have percolated through to Capitol Hill, and last month Congress approved an appropriations bill for NIH (which has since been vetoed by President Ford), which contained \$3 million to establish a research programme on job-related cancers.

With discoveries of new occupational and environmental carcinogens—such as the link between vinyl chloride gas and liver cancer in plastics workers—continuing to flood in, the death rate from cancer continuing to increase (it rose dramatically in the first six months of 1975 in the US, but the increase may be a statistical quirk), and the cancer programme continuing to occupy a politically favoured position in the federal budget, it seems inevitable that there will be a renewed political debate about the structure and scope of the cancer programme this year. □

DELEGATES from the contracting parties to the London Dumping Convention 1972, along with observers from other countries and interested international organisations, met for the first time last month in London to choose an appropriate organisation to undertake its secretariat duties and to begin preparations for its first substantive meeting later this year.

The world-wide Convention—its full title is the Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, London 1972—has been signed by 54 states, 22 of which have ratified it. The Convention prohibits the dumping at sea of certain dangerous substances, including mercury and cadmium and their compounds, organohalogen compounds and highly radioactive materials, and subjects the dumping of a large number of other substances to stringent control by the issue of specific permits by national authorities.

The conference agreed to a joint proposal from Britain and Mexico that the secretariat's work should be given to the Inter-Governmental Maritime Consultative Organisation (IMCO), the London-based UN agency with over 90 members which has until now administered conventions regarding marine pollution from ships and operated in the fields of marine safety and navigation.

The conference set up a working committee to examine the plan, and also agreed that the first substantive meeting of contracting parties to the Convention should be held in September.

- The enforcement from the beginning of January of further provisions of the Control of Pollution Act 1974, which covers waste disposal and reclamation, noise, water and air pollution, has coincided with the Department of the Environment's latest survey revealing a further slight fall in the extent to which the UK's rivers and canals are polluted.

A total of 840 miles of river and canal is now classed as "grossly polluted", a reduction of 46 miles. The improvement has increased marginally the lengths of river classed as "poor quality requiring urgent improvement", but at the other extreme an extra 160 miles have been added to the lengths considered free from pollution.

The survey is based on tests carried out in 1973. The improvement on the previous survey in 1972—the first was made in 1970—was less marked in tidal rivers and canals than in non-tidal rivers. And altogether a total of 182 miles was downgraded.

- The reports of three separate committees examining lead levels in food were published last month when the Working Party on the Monitoring of Foodstuffs for Heavy Metals carried in its fifth report (*Survey of Lead in Food: First Supplementary Report*) two appendices containing the advice of the Food Additives and Contaminants Committee and of the

## Around Britain



toxicity sub-committee of the Committee on Medical Aspects of Chemicals in Food and the Environment.

The Working Party, whose second report, in 1972, was also a survey of lead in food, concluded that the amounts of lead in the average diet in the UK had not increased since then, and that the mean lead concentration in canned infant foods had significantly decreased. The toxicity sub-committee, which advises the Food Additives and Contaminants Committee and various government departments on the toxicological aspects of the use or presence of additives and pollutants in food and the environment, considered that the present intake of lead from food in the UK was unlikely to constitute a hazard to the health of the general population.

The Working Party's surveys, involving the analysis of some 6,000 samples of foodstuffs and nearly 12,000 samples of drinking water, showed that the average consumer's lead intake was less than half the 'provisional tolerable weekly intake' of 3 mg for adults, published by the FAO/WHO.

But the Food Additives and Contaminants Committee, although endorsing the underlying view, recommended reductions in the maximum permitted amounts of lead in food in its separately published *Review of the Lead in Food Regulations*. Margins of safety were not as great as could be desired, the

committee argued. It wanted ministers to enforce a limit of one part per million for most foods, a lower limit for baby foods, an end to the exemption from all limits of fish in which lead occurs naturally, and extra efforts to cut the amounts of lead in tinned foods.

- Just a couple of weeks after the announcement that Lord Beswick would head the new publicly owned corporation British Aerospace when it is formed—an appointment that attracted some Opposition criticism because he has not held an executive position of any sort in industry—the Labour Government named the board members of the new British National Oil Corporation (BNOC) whose first task will be to organise the structure of the state oil undertaking. And on the list so far is not a single senior oil industry management executive.

Indeed, the only man with any remotely practical experience in the area seems to be Mr Denis Rooke, chairman-designate of the British Gas Corporation. But his appointment, like that of the chief executive of the Shetland Island Council, drew speculation about conflicts of interest as well as about BNOC's possible influence over British Gas. And the inclusion of Lord Balogh as deputy chairman, a part-time post, has raised questions about BNOC's independence from government interference, because although he has retired as Minister of State at the Department of Energy (headed by Mr Anthony Wedgwood Benn), Lord Balogh remains Mr Benn's special adviser.

The chairman of the board, which also includes a lawyer, a trade union leader and several civil servants, is Lord Kearton, formerly head of Courtaulds.

- Dr Walter Marshall, the Director of Harwell, the Atomic Energy Research Establishment, is relinquishing his position (though not, apparently, his office) there to become deputy chairman of the UK Atomic Energy Authority (UKAEA) on Mr Frank Doggart's retirement. He will retain his other major post, that of chief scientific adviser to the Department of Energy, except when it comes to nuclear matters, which are the province of the UKAEA chairman, Sir John Hill.

The move, announced by Mr Benn, marks a large promotion. Dr Marshall was, at 43, the most junior member of the UKAEA Board; he now has responsibility for scientific and technical policy throughout the organisation. Dr Lewis Roberts will take his place as director of Harwell.

AS THE Council for Mutual Assistance (Comecon) countries considered the end of their 1971-75 Five-year Plans and formulated their plans for the coming five years, a whole series of meetings, conferences and discussions were held of the Standing Comecon Commissions. Plans have been put forward on such diverse matters as the unification of standards for industrial production, the approval of new drugs to be used in the treatment of malignant tumours, the division of labour between the participating countries in the manufacture of electronic components, and specialisation and co-operation in the manufacture of instruments for the artificial insemination of cattle.

For the first time, the new plans will include the construction of joint industrial projects by a number of member countries. And the non-European members of Comecon are not forgotten. Within the framework of the existing scheme for the integration of information systems, Czechoslovakia and Hungary are to provide information systems for Cuba, and the Soviet Union will provide Cuba and Mongolia with industrial, agricultural, and transport equipment in return for basic exports and raw materials.

● The new Polish five-year plan, as laid down by the Seventh Congress of the Polish United Workers' Party which met in Warsaw from December 8-12, envisages a considerable expansion of investment in science. This is despite the fact that the growth of the Polish economy in the five years of the Gierek regime has been facilitated by foreign borrowings which, in bringing

demands for interest payments, will make higher prices and food shortages likely over the next five years.

## Comecon diary

from Vera Rich, London



Expenditure on both science and technology is put at 200,000 million zloty, after 115,000 million in the previous five years. Special emphasis is to be placed on research into the more efficient use of fuels, raw materials and water resources, improving the technology of the coal and copper industries, the introduction of aluminium production from native ores and the production of new materials for the electronics industry. The application of science to agriculture, public and occupational health and child care are also mentioned.

According to Sylwester Kaliski, the Minister of Science, Higher Education and Technology, the plans will be im-

plemented through a new scheme which, with cooperation between scientists and administrators, will lay the foundation of a "strategy for scientific research and technical advance, a plan composed of government programmes, departmental problems and basic research". This, he said, would unify the whole activity of research and its practical implementations in Poland.

The Polish Academy of Sciences is to play an important role in the research part of this programme, and it is introduced also to involve the universities.

● In the German Democratic Republic's economic plan for 1976, which (if successful) will result in a 5.3% increase in the national income, a 6% increase in the production of industrial goods and an improvement in labour productivity of 5.5%, the plan for science and technology envisages more than 160 specific tasks to be fulfilled on time and to a high standard. The "tasks" cover a wide range of applied science, from improved fuel economy in power stations to rationalisation of the washing-powder industry.

The attainment, or otherwise, of the proposed targets may itself be somewhat difficult to evaluate, however. According to the East Berlin Central Statistics Board, a number of enterprises in the GDR have been "interpreting in their own special way" their economic results for 1975, so that in many cases the figures submitted make the drawing of any sound economic conclusions problematic. The board urges exact calculation, rather than "interpretation", of the figures.

## Indian states attack population growth

from Our Correspondent, Jullundur

AFTER more than two decades and the expenditure of thousands of millions of rupees, India's family planning programme has achieved only limited success. But Draconian measures proposed by two state governments may well change all that through a revamped and much more severe stick-and-carrot policy to encourage sterilisation.

Taken up officially in 1952, the Indian birth control programme started off modestly, but only received its first big push after the publication of the 1961 census data, which showed a higher-than-expected rate of population growth. The programme has nevertheless made only slow progress. According to official figures, the present birth and death rates are 36.6 and 16.9 per thousand respectively. Due to improvement in health services, the

death rate has fallen and is expected to go down even further. But the slow decline of the birth rate can be attributed to a number of factors. Paucity of funds, inadequate communications and lack of official enthusiasm at lower levels have proved obstructive; but poverty, malnutrition, ignorance, superstition and illiteracy are the more formidable hurdles. There have been suggestions that new families be allowed to have no more than two children. But so far the government has steered clear of introducing compulsion into the programme, even though the population increases each year by an amount equivalent to the population of the whole of Australia.

The state governments of Haryana and Punjab, however, have decided on steps which could spell the beginning of a new trend. The Haryana government has announced a series of measures to promote smaller, two-children families by means of incentives and disincentives. The state has set itself a target of reducing its net

rate of population growth to zero before 1985.

The incentives include cash prizes worth 100,000, 75,000 and 50,000 rupees, which will be awarded each year to three districts in the state which capture top spots in family planning performance over and above their allotted targets. Factories in the state, where all 'eligible' workers accept sterilisations after two children, will qualify for benefits from a 'Labour Welfare Fund', and the best village-level governing bodies in each district will also be eligible for cash bonuses. All children of 'eligible' government employees will be entitled to reimbursements of medical expenses up to the age of 5; thereafter only two of them will be given this facility. Employees who already have more than two children will only receive medical reimbursements if they are sterilised within a year of the date of enforcement of these decisions.

The disincentives are aimed primarily at government employees with



more than two children who fall into the category of 'eligible' couples. Unless they get themselves sterilised within 12 months, they will not be allotted government accommodation, or, if they are already in possession of government accommodation, they will be charged standard rent; they will not be allowed any house-building advance, or loans for buying wheat or vehicles; and on being transferred, they will receive travelling allowances only for two of their children. New recruits into Haryana government service will have to undertake not to produce more than two children. A third child will render them liable to dismissal.

Days before the Haryana government decisions, the Punjab government had announced similar measures to curb the rate of population growth in that state. Among other things, women employees in Punjab would no longer be provided the facility of maternity leave and other related benefits beyond the second child. □

## Commission foresees 200-mile limit

THE European Commission has recently disclosed details of proposals regarding European fishing policy, grasping in the process the nettle that the problem of 200-mile fishing limits represents.

The plans, which now require a decision from the nine member governments, envisage a time when 200-mile fishing limits are approved—most likely at a forthcoming session of the UN Law of the Sea Conference. Member governments are enjoined to formulate a common position on the issue.

The Commission emphasises the need for conservation of fish resources in its proposals, and this would probably involve setting catch quotas. The inland waters of each of the nine member states should, it says, be opened to fishermen from other member states

(this is due to happen after 1982), and argues that special arrangements will have to be made to protect inshore fishermen and preserve fish stocks when limits are extended to 200 miles.

Quotas would have to be negotiated with non-member countries like Spain and Norway, the Commission says, and access terms for Community fishermen in their waters would also have to be agreed.

In Norway itself, meanwhile, the minister in charge of questions relating to the law of the sea, Mr Jens Evensen, has spoken about the sort of agreements he foresees regarding conservation and quotas. He was indicating new guidelines for Norway's negotiations with 15 countries about the establishment of its proposed 200-mile economic zone. The total catch, he said, should be allotted to the various countries with a clear preference for the coastal states. And he described the EEC fisheries policies as a "heavy burden for Europe". □

OPEN season on the Food and Drug Administration (FDA) is, as usual, in full swing; cannon to right of them, cannon to left of them, volley and thunder, in the "health food" magazines, in *Science*, where the anti-everything editorial staff have long used FDA as a favourite target, and even in *Nature*.

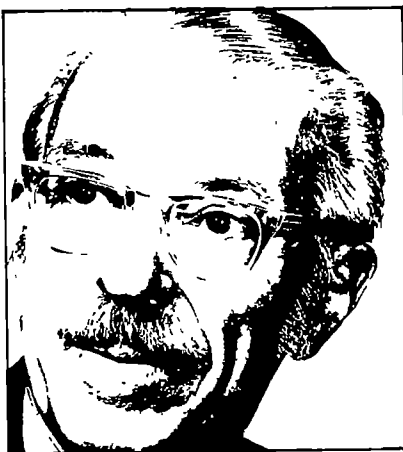
A new report on cyclamates says that "a blue-ribbon scientific panel" tentatively concluded on December 10 (with one dissident) that more than 20 new studies with animals failed to show cancer, birth defects or genetic damage. Now what happens? If cyclamates are put back in soft drinks, the Center for Lawyers in the Performing Arts will erupt in rage, aided by FDA's in-house consumerist, who will inject chick embryos with cyclamates. The embryos will die. Chickens, unlike dogs, are expendable. (But what dog ever laid a nice, fresh breakfast egg?) The FDA will be accused of collaborating and conniving with the pharmaceutical industry to put a poison in our food.

However, if cyclamates are not put back in soft drinks, the Toby Belch Society, or some other watchful guardian of the obese, will protest angrily. The FDA will be accused of plotting and conspiring with the sugar industry to keep a harmless sweetener from people who need it in reducing diets. The FDA must indeed feel grateful for the existence of cyclohexylamine, a metabolic product of cyclomates that may give the regulators a little breathing spell while it is further investigated. Few of the critics will bother to read the Federal

Food, Drug and Cosmetic Act, which gives very specific directives for evaluating applications for the use of food additives.

Some confusion in this general field undoubtedly arises from lack of understanding of the "GRAS"

## The policeman's lot



THOMAS H. JUKES

(generally recognised as safe) list. This list, which is currently being overhauled, includes about 670 substances recognised in 1958 as suitable for food, compiled from FDA food standards, from State regulations, and from lists of substances "known to have been used in food for some years without reported adverse effects." Long custom rather than scientific investigation led to the acceptance of most of these as (or in) foods. Undoubtedly, many of them would not "make it" if intro-

duced as "new" food additives. The FDA appears to be getting blamed for allowing bacon, containing mutagenic substances, to be sold. Just who got the idea of soaking meat in nitrates to preserve it is not clear, but the practice certainly antedates the FDA, and the present century. For that matter, the treatment of meat with smoke adds carcinogens to the diet, and this process must have originated in the caves of our remote ancestors.

The FDA points out that, for all food additives, new information casting doubt on safety will lead to "necessary measures provided for in the law to be undertaken to protect the public health." Actually, some additives, such as safrole, have been quietly dropped. There are no provisions, of course, for using new information of a positive nature, such as the increase in mean life span of mice, and the partial suppression of carcinogenicity of certain aromatic amines, both reported for the antioxidant butylated hydroxytoluene (BHT), whose name is enough to make it suspect, regardless of its properties.

Meanwhile, FDA is still ducking bullets on other issues, as described in *Nature* (November 20). Charges of "industry favouritism" by disgruntled employees make great headlines, and nothing is duller than a rebuttal. Industry, of course, says it is strait-jacketed by FDA. The pushers of the quack cancer remedy "Laetrile" say they are, too, as they lie a-basking in the sun, south of the border, down Mexico way. The policeman's lot is not a happy one.

# correspondence

## Higher education

SIR,—May I comment on two related articles on higher education which have appeared in recent issues of *Nature* (November 13 and 20)? The question of whether universities should be concerned with "education" or with professional "training" is raised in relation to the balance of faculties and the cost to the taxpayer.

In my view there is a need for two distinct types of higher educational institution. One of these, traditionally universities, should be principally concerned with scholarship and furthering the bounds of human knowledge, and with passing on the fruits of such knowledge and research, through teaching, to any who are sufficiently interested and wish to receive it. Examinations, if they are appropriate here at all, should not take the form of professional qualifications, but should rather be used for selecting academics to continue the work of the university.

The other type of institution should have the specific function of training for professional qualifications. Such an institution might be a polytechnic where the teaching should be geared to qualifying examinations for professional men expecting to practise in medicine, the law, sciences, engineering, business management or whatever. The teachers should have had some years of practical experience and they should constantly refresh that experience on a sabbatical principle or by periodical exchanges with practitioners. Research work should be aimed at practical application in response to the needs of industry, hospitals and so on.

Universities should be reduced in number and much reduced in size, but the balance of faculties should certainly be maintained. This would provide an environment for the broad education of relatively few who are academically inclined and who would be expected to achieve the highest standards of excellence in scholarship, imagination and innovation.

Polytechnics could afford to be more specialised and might benefit from it. In order that the trainees should not suffer from a lack of education in the broader sense, the polytechnics should allocate some course time to a variety of subjects for which the universities could send lecturers and set up

seminars. There would be no examinations in these extra-curricular subjects and a wide choice should be offered.

The courses offered by polytechnics should ensure that a sound basis of theory and practice is achieved, and the chartered professional bodies should have a considerable voice in the curricula and in the form and content of the qualifying examinations. In some professions where developments are rapid, it might be desirable to insist on further training and examination at intervals to ensure that high professional standards are maintained throughout a career.

Developments in the future along these lines might ensure better academic research, better professional standards, and give better value for the taxpayer's contribution to higher education.

Yours faithfully,

A. C. MASON

Wirral, Merseyside, UK

## The protein gap

SIR,—Man has always desired simple answers to the complex problems of the world; to reduce everything to a common denominator. Many have done this with regard to nutritional problems, claiming over the past twenty years that protein was the only answer. As Drs Waterlow and Payne point out in their article "The Protein Gap" (November 13), this is not true. Unfortunately they then fall into the same error by concluding "the protein gap is a myth and what really exists . . . is a food gap and an energy gap". The new simple answer for them is energy.

Protein-energy malnutrition, be it kwashiorkor or marasmus, has never been, except in the rarest of cases, a single nutrient deficiency disease. It has always involved the lack of vitamins and minerals as well as energy and/or protein and all the other factors required for good nutrition.

To talk only of energy and protein as the FAO/WHO report did, and as the authors do, is meaningless. To suggest merely that more of traditional diets is all that is needed is, in most cases, no solution to nutritional problems. Many of these diets were shown to be inadequate by early workers such as McCarrison and they continue to be shown so in current studies.

Nutrition deficiencies are encoun-

tered primarily in populations with a limited choice of foods. Adequacy is best assured through the use of a wide variety of foods having complementary patterns of nutrients, as has been pointed out by the US National Academy of Sciences. This means improving traditional dietary patterns.

Until man is willing to reckon with the complexity of the nutrition problem and to deal with it as such, he is doomed to repeat the errors of the past.

Yours faithfully,

WALTER J. BRAY

Department of Food Science,  
University of Reading, UK

## Human consumption

SIR,—The statistic of the Potato Marketing Board quoted in *Nature* (December 11, page 484), and quoted here in full,

"Contrary to the trends in all other countries in the European Economic Community the rate of movement into human consumption in Great Britain increased during the three previous seasons (and shows a provisional offtake for 1974/75 of 222 lb per head per annum)",

may suggest spud-deficiency to you, but it may imply an alarming increase of cannibalism to others.

Yours faithfully,

JOEL J. LLOYD

National Academy of Sciences,  
Washington, DC 20418

## Peace Prize

SIR,—Your leader (October 16) concerning the awarding of the Nobel Peace Prize must be the ultimate expression of English diplomacy. Tell me, what better example anywhere of peaceful struggle for freedom is there than Sakharov's? No one would have to lose any freedom themselves to satisfy his requests, and unlike some prizewinners he would not have to lay down his gun to pick up the prize. Yet you say it shouldn't be given because it might be offensive to the bureaucracy of the Soviet Union. What utter hypocrisy for such an editorial to appear at such a time.

Yours faithfully,

FRANK SORENSON

Corvallis, Oregon,  
USA

# news and views

## High resolution interferometry

from F. G. Smith

THE angular accuracy of radio astronomical maps of celestial objects has progressed beyond all optical measurement, but it has reached a natural limit. This limit is provided by the available intercontinental distances between the component radiotelescopes used in Very Long Baseline Interferometry (VLBI). The observations reported on page 17 use multiple baselines between the 100-m radiotelescope in Germany and three American radiotelescopes, at Green Bank, Owens Valley, and Fort Davis. At a wavelength of 2.8 cm a source diameter of only  $0.25 \times 10^{-3}$ " can be resolved by this multi-element interferometer, which uses all of the six independent baselines between the four telescopes. The combined efforts of 13 radioastronomers, and doubtless many supporting staff, at these telescopes now give us a map of the central radio source in the galaxy NGC1275, well known optically as a Seyfert galaxy with very large internal velocities. The radio map only covers the nucleus of the galaxy, with a total extent of 5 pc, but the resolution is 0.1 pc. Longer

wavelength radio emission, as mapped by the 5-km radiotelescope at Cambridge, comes from much more extensive regions, while the whole galaxy extends to a diameter of more than 50 kpc. It is the very core of the galaxy that is being explored in this new map.

The technique of VLBI does not give the same full and unambiguous map as does aperture synthesis. For example, the nucleus of NGC1275 is elongated, with components whose sizes, shapes and relative dispositions are now fairly well determined: but it would be equally possible for the whole map to be rotated by  $180^\circ$ . This is of no consequence in the physics of this radio source, which is known to be active on a very short time scale. Most of the radio emission has appeared during the last 15 yr, since the time that measurements started on short wavelengths.

Rapidly changing radio sources present further problems in interpreting VLBI measurements. If a single baseline is used for measuring an apparent

source diameter, the source can appear to grow or shrink alarmingly rapidly.

An effect of this sort has even led to the suggestion that some components of radio galaxies are expanding outwards with velocities greater than that of light. Such an interpretation requires the various components of a source to retain their relative intensities, and to change only their relative positions. The isolation of several small components within the nucleus of NGC1275, which must be the seat of the large intensity changes observed since 1960, suggests that it is premature to interpret any changes of configuration of radio galaxies as due to "super-light" velocities.

The physical processes in the nucleus will provide much food for thought. Large parts of the galaxy are moving with relative velocities of over  $3,000 \text{ km s}^{-1}$ . Many theorists consider that the kinetic energy of this motion comes from an explosive event in the nucleus, where the radio emission now demonstrates the active generation of high energy particles and magnetic fields.

## Behaviour of the ionosphere and plasmasphere during geomagnetic storms

from T. B. Jones

THE interaction of the solar wind with the Earth's outer environment involves complicated processes many of which are not yet fully understood. The geomagnetic and ionospheric storms produced by enhancements of the solar wind are particularly interesting since they give rise to major changes in both the ionosphere and magnetosphere. These in turn, disrupt long range radio communications and navigation systems which depend on the reflection of radio waves from the ionosphere.

Solar disturbances frequently produce an increase in the velocity and concentration of the particles of the solar wind. When this enhanced wind

reaches the Earth's magnetosphere it produces several phenomena which include modification of the Earth's field, increased auroral activity and marked changes in the electron density distribution of the ionosphere. The arrival of the enhanced solar wind at the magnetosphere boundary increases the compression of the geomagnetic field which is observed as a sudden commencement geomagnetic storm on the Earth's surface. Simultaneously, the number of energetic particles in the magnetosphere trapping regions increases as particles are injected into the magnetosphere by transport across the magnetic shells. The increased ring current due to the particle enhance-

ment is responsible for the main phase of the storm. A detailed account of the dynamical processes which govern the behaviour of the trapped particle belts can be found in *Particle Diffusion in the Radiation Belts*, (Schulz, Lanzerotti and Roederer, Springer-Verlag, 1974).

Changes of shorter duration known as 'polar substorms' can occur in the polar magnetic field. These disturbances are a feature of the 'auroral oval' which corresponds to the region where the field lines that leave the Earth's surface stretch out into the magnetotail. The role of the north-south component of the interplanetary magnetic field in substorm processes

has been the object of some controversy. It is generally thought that the southward turning of the field leads directly to a series of growth processes which trigger the substorm. On the other hand measurements in Alaska indicate that substorms occur frequently even when the field component is directed northwards. This conflict has been resolved by Akasofu (*Nature*, **286**, 191; 1975) who suggests that the occurrence of a substorm depends neither on the field direction nor on its rate of change, except that if a steady northward field ( $>6$  h) is established then no substorms are observed. During these events energetic particles travelling along the magnetic field lines penetrate into the lower ionosphere where they produce ionisation enhancements in both D and E regions which greatly affect the propagation of radio waves through these regions. It has been suggested that even lower levels of the atmosphere are influenced by geomagnetic storms. Olson (*Nature*, **257**, 113; 1975) has found that at tropospheric heights, the 500 mbar vorticity index, which is a rough measure of cyclonic activity in the Northern hemisphere, decreases during the main phase of a storm.

Storm-induced disturbances are not confined to polar latitudes and the F region of the ionosphere is affected even at the magnetic equator. Both increases and decreases in this layer's electron density are observed depending on the particular storm event. The detailed mechanisms of F region storms have been discussed by Rishbeth (*J. atmos. terr. Phys.*, **37**, 1055; 1975) and changes in thermospheric circulation are shown to be a major factor in producing the observed ionospheric disturbances. In principle, both positive and negative storms can be caused by circulation changes though it is likely that other factors such as electromagnetic drift, ionosphere-magnetosphere plasma flux and the effects of propagating gravity waves have important roles especially at low latitudes. Storm-induced exchanges of ionisation between the ionosphere and protosphere have been observed by Soicher in a recent satellite experiment (page 33, this issue).

In view of the complexity of the storm processes there is an urgent need for further measurements of particle fluxes, magnetic field variations and ionisation changes. An international cooperative effort to obtain these observations will be made during the forthcoming International Magnetosphere Study (IMS), period 1976 to 1978. A wide range of ground based and space vehicle techniques will be deployed in a coordinated programme to study the magnetosphere and its

influences on other regions of the Earth's environment. □

## Weak gamma-ray bursts

from A. C. Fabian

SOURCE counts are widely used by astronomers working in all parts of the spectrum, and appear under various names and guises. Comparisons of the number of faint sources with brighter ones can yield information on the geometrical distribution of sources in space, their evolution with time, cosmological effects and the distribution of absorbing material. This information is gained without any precise knowledge of the distances to the sources. Unfortunately, however, source counts do not give unique solutions to problems. This is mostly due to the fact that there is always some spread in the intrinsic luminosities of the sources.

The latest category of events to be counted are cosmic gamma-ray bursts. These are the enigmatic flashes of gamma radiation first detected by the Vela satellites. The only facts really known about them are that they show complex structure on time scales of hundredths of seconds to tens of seconds, their spectra somewhat resemble those expected from thermal bremsstrahlung from regions of temperature  $\sim 10^8$  K, and their origins lie outside the inner Solar System. Counts of the more intense bursts detected by the Vela and other satellites, with integrated fluxes greater than  $\sim 10^{-4}$  erg  $\text{cm}^{-2}$ , suggest that they are relatively uniformly distributed in space. The rate is such that weaker events ( $\sim 10^{-7}$  erg  $\text{cm}^{-2}$ ) are predicted to occur several times per hour. Such a prediction is testable by balloon-borne detectors, and Bewick, Coe, Mills and Quenby of Imperial College London have recently reported the results of a series of flights (*Nature*, **258**, 686; 1975).

Only one event was observed, of size  $1.2 \times 10^{-7}$  erg  $\text{cm}^{-2}$ , in 13.5 h of observing time. This is a significantly different rate to that predicted. In the authors' opinion it suggests that a uniform source distribution can be excluded. Such a result might be expected if the sources are galactic, for then there might be fewer weaker sources observed in directions perpendicular to the galactic plane. Cosmological effects—principally redshift—might be invoked if the sources of gamma-ray bursts are extragalactic. Some caution is appropriate in interpreting the results, however, since

event rates are being compared between different detectors of somewhat uncertain apertures, efficiencies and energy ranges. Consequently there are errors in the integrated fluxes that are difficult to estimate. The Imperial College detectors appear to be sensitive enough to avoid limitations due to the transient nature of the bursts. This would only become serious if a substantial fraction of the bursts had timescales of 10 s or more, but there is at present no indication of this being so.

There are undoubtedly many other groups building or flying balloon-borne detectors to monitor weak gamma-ray bursts. It is hoped that these will confirm the first results and will eventually define the shape of the size distribution of weak gamma-ray bursts. The rare stronger bursts will probably only be available for study by satellite detectors. We are still no closer to a theoretical interpretation of gamma-ray bursts, although it is notable that the number of theories proposed to explain them is now lagging behind the number of detected events. □

## Fish that change sex

from John R. Krebs

To a narrow-minded mammal the sex life of the Blue-headed Wrasse, a tropical reef fish, may seem odd. The Blue-headed Wrasse occurs in two colour phases: smaller fish are highly variable in colour and may be either male or female; while larger, older individuals are always blue and green with two vertical black stripes, and are invariably male. Some individuals go through the first (initial phase) colour pattern as a female and turn into a male when they reach the striking terminal colour phase. Other individuals go through both phases as a male. The transition from initial to terminal colour phase and the accompanying sex change can be achieved rapidly. If all the terminal phase males are taken away from an area of reef, initial phase males or females soon start to change colour and become terminal males.

Most of the fertilising of females is done by terminal males. They set up temporary territories at around midday at a specific spawning site on the reef. Here they wait for females to turn up, while the smaller initial phase males mill around in large numbers (often in hundreds). When females arrive at the spawning area they prefer to mate with the territorial terminal males, and a lucky male may mate up to 100 times a day. Initial phase males sometimes manage to fertilise a

NITROGEN fixation commonly occurs in bacteria only when the oxygen tension is low either because the nitrogen fixer is an obligate anaerobe such as *Clostridium* or because the aerobic organism, *Azotobacter* for example, has in addition to the normal ATP synthesising system, a very active electron transport chain that operates without conserving energy. This results in a micro-aerobic environment around the site of nitrogen fixation (Postgate, in *Biological nitrogen fixation*, edit. by Quispel, A., 1974) and brings about a respiratory protection of the nitrogenase.

The nitrogen-fixing blue-green algae have a particular problem since their photosynthesis leads to oxygen evolution; they may fix nitrogen only at low oxygen tensions and low light intensity (non-heterocystous forms) or they may segregate their nitrogenase into special compartments (the heterocysts) to which the fixation of nitrogen is normally confined. Only under artificial conditions of anaerobiosis do the ordinary vegetative cells of heterocystous blue greens have active nitrogenase.

Heterocysts are specialised in that they seem to lack the oxygen-evolving component of photosyn-

thesis, photosystem 2. They may also remove unwanted oxygen diffusing in from the environment by the use of an active respiratory chain. Thus a low oxygen tension could be maintained at the site of nitrogen fixation.

The absence of photosystem 2 has been deduced from the lack of accessory pigments, from the low yields of chlorophyll fluorescence and from the inability of heterocysts to fix carbon dioxide (Stewart, *A. Rev.*

## Nitrogen, oxygen and manganese

from F. R. Whalley

*Microbiol.*, **27**, 283; 1973). The association of photosystem 2 with manganese has also been widely documented (for a review see Cheniae, *A. Rev. Pl. Physiol.*, **21**, 467; 1970) although the evidence has always been indirect. Recently Tel-Or and Avron (*Proc. third int. Congress on Photosynthesis*, 569; 1974) reported isolation of a heat stable manganese-containing compound of small molecular weight which, when added back to depleted particles isolated from a blue-green alga (*Phormidium luridum*) restored oxygen evolving ability, thus

providing direct evidence of a role for manganese in O<sub>2</sub> evolution.

The lack of photosystem 2 in heterocysts in another blue-green alga (*Anabaena cylindrica*) is now further substantiated (Tel-Or and Stewart, *Nature*, **258**, 715; 1975), following an examination of the distribution of <sup>54</sup>Mn between vegetative cells and heterocysts isolated from cultures grown with <sup>54</sup>Mn. This showed a correlation between the presence of <sup>54</sup>Mn, the Mn:chlorophyll ratio, and the ability of the isolated cells to evolve oxygen. The clear conclusion is that heterocysts of these nitrogen-fixing algae are deficient in manganese, that they lack a functional photosystem 2 and therefore cannot evolve oxygen. Heterocysts are thus specially adapted for nitrogen fixation. By contrast, the vegetative cells accumulate manganese and evolve oxygen but do not appear to fix nitrogen. It is of correlative interest (Anderson *et al. Biochem. biophys. Res. Commun.*, **17**, 685; 1974) that fractions of isolated chloroplasts which have been depleted in photosystem 2 also become depleted in bound manganese whereas those enriched in photosystem 2 become enriched in manganese.

female's eggs by dashing into a territory at the last moment. More often, they chase an arriving female as a group and achieve a sort of piscine gang bang.

The facts are remarkable enough, but what is the evolutionary explanation for this strange reproductive system? Warner, Robertson and Leigh (*Science*, **190**, 633-638; 1975) develop an idea of M. T. Ghiselin, who argued that natural selection will favour sex change whenever the reproductive potential of one sex increases rapidly with age while the other remains fairly constant. In this circumstance it will be advantageous to start life as the sex which does not benefit from being older (or to put it another way, does not suffer from being young) and switch to the other sex at a later stage. This is what seems to be happening in the Blue-headed Wrasse. Terminal males have an enormous reproductive potential, while initial males have a low mating success, so an individual can get the best of both worlds, by starting off as a female (young females do not suffer any penalty for youth) and switch to being a male at a later stage when it can successfully defend a mating territory. But why do some individuals go through the initial phase as a male and not a female? Warner *et al.* suggest that in large populations, where many females arrive simul-

taneously at the spawning area, the opportunities for an initial phase male to grab the odd fertilisation are high enough to make being a male in early life about as profitable as being a female. Their data show that the daily mating rates of an initial male and a female in a large population are about the same. In a small population, however, there are fewer chances for the initial males. There are rarely enough females at the spawning site to enable an initial male to steal a fertilisation. As this account would predict, initial males are much rarer in small populations than in large ones. Also, in other wrasse species that always occur in small groups, initial phase males are always rare.

If, as Warner *et al.* suggest, sex change is an optimal strategy when there is a big advantage in being old in one sex but not in the other, why don't mammals such as seals, deer and baboons, in which the older males monopolise females, change sex like the wrasse? The authors suggest that one or more of three factors may prevent them from doing so: the physiological cost of changing sex in an animal with internal fertilisation, the rigidity of the mammalian sex determination system, and the fact that in mammals competitive experience as well as size is important in determining the reproductive superi-

ority of older males. Obviously this experience would be lost if a male spent its youth as a member of the opposite sex. □

## How old are cosmic rays?

from J. J. Quenby

WHILE recent progress in observational astrophysics has been rapid, the answer to the fundamental question as to the origin of cosmic radiation remains elusive. This flux of relativistic, charged particles populates the Galaxy with an energy density sufficient to influence significantly the dynamics of the whole system, but we do not know whether relatively near objects like the Crab Nebula or distant, energetic objects like strong radio galaxies constitute the prime source. An important clue would lie in an ability to 'read' the <sup>10</sup>Be 'clock' in the cosmic ray flux. The light elements Li, Be and B are rare in cosmic material and should not be present at the source of particle acceleration. By knowing production cross sections for the light elements in collisions between nucleons and atoms it is possible to calculate the amount of material through which the average



cosmic ray must have passed in order to produce the observed elemental abundance in the radiation. Then by finding the amount of radioactive  $^{10}\text{Be}$ , which has a half-life for decay of  $1.5 \times 10^6$  yr, relative to other light nuclei abundances, the actual cosmic ray age is deduced. If cosmic rays are not of Universal origin, that is to say, if they do not fill extra-galactic space as they do the Galaxy, then they must be confined either to the region of the galactic disk or galactic halo. The latter is an approximately spherical region, including the stellar disk, maintained by magnetic fields which trap charged particles and cause radio emission. By cosmic ray life we mean their time to diffuse or leak from the confinement region, although some are lost catastrophically by nuclear collision with the material of the trap. Present estimates allow a cosmic ray life of a few  $10^6$  years if confinement is to the disk, or alternatively a life in excess of  $10^7$  years if confinement is to the halo.

Satellite data on the  $^{10}\text{Be}$  abundance obtained by Garcia-Munoz, Mason and Simpson of Chicago University (*Astrophys. J. Lett.*, **201**, L141–144; L145–148; 1975) suggests a halo rather than a disk confinement. These workers employed small charged particle telescopes mounted on the IMP-7 and IMP-8 satellites which sampled interplanetary conditions. Isotopes were separated to an accuracy of 0.4 atomic mass units by looking at energy loss in two solid state detectors and residual particle energy in a crystal scintillator. They found few or no  $^{10}\text{Be}$  particles although  $^7\text{Be}$ ,  $^9\text{Be}$  and  $^{10}\text{B}$  events were detected.

If accepted, these interesting results will ease a related problem, concerned with the anisotropy in galactic arrival directions of the cosmic ray flux. Although an asymmetry of roughly 0.01% seems to be present, according to Imperial College measurements, the demands on the magnetic scattering process for a disk confinement model are severe. The short total path length requires a large amount of scattering to keep the anisotropy small and this in turn causes a large energy drain as the charged particles interact with the scattering centres. Halo confinement means less scattering and hence less energy drain. Alternatively the small anisotropy would be thought to imply a universal cosmic ray flux.

Although the Chicago group have certainly developed a high-quality experimental technique, some difficulties remain with the result. To date Balloon groups at Goddard Space Flight Center and the University of New Hampshire have also achieved isotopic resolution in the light element range, but they report  $^{10}\text{Be}$  fluxes twice or four times those suggested by the satellite data. It

could be, however, that interactions above the balloon or magnetospheric processes have influenced the balloon results. Another point is that the lifetime deduced depends on assumptions as to the cosmic ray source spectra and these have to be obtained from trial models for the motion and interactions of cosmic rays in the confinement region. Furthermore, the final result clearly depends on the allowance made for solar modulation in the interplanetary medium. Diffusion and energy loss effects due to the interplanetary magnetic field tend to enhance the amount of  $^{10}\text{Be}$  observed relative to neighbouring nuclei. At present we do not know whether the total cosmic ray flux at Earth is close to the interstellar value or as much as six times lower. If the modulation is small then even the Chicago  $^{10}\text{Be}$  flux would be consistent with disk confinement and life of a few  $10^6$  yr for cosmic rays. Thus our final reading of the  $^{10}\text{Be}$  'clock' will depend on further studies to determine the scattering power of the interplanetary medium. A definitive answer probably requires a direct measurement of the interstellar cosmic ray flux, but this may not be achieved with planned missions in the ecliptic plane to Uranus and beyond. Indeed the lack of noticeable change in the cosmic ray intensity on the way out to Jupiter, as recorded by Pioneers 10 and 11, is one of the puzzles which illustrate our lack of understanding of modulation. Perhaps an off-ecliptic mission to solar polar latitudes where cosmic ray access is thought to be easier is the correct experiment for determining the  $^{10}\text{Be}$  abundance. □

## The Red Queen dethroned

from A. Hallam

IN recent years there has been an increasing tendency in some circles to investigate the evolutionary record of fossils in terms of general rules and processes without regard to specific causes operating on specific taxa. Van Valen (*Evol. Theory*, **1**, 1; 1973) has made a notable contribution in this respect by applying the survivorship curve technique of population biologists to the study of extinction rates for numerous fossil taxa. Van Valen claimed to demonstrate a general approximation to linearity in his curves, which are cumulative frequency distributions of taxonomic durations with logarithmic ordinates. This led him to propose a new evolutionary "law", which, in brief, states that within a relatively homogeneous higher taxon,

subtaxa tend to become extinct at a stochastically constant rate. As an explanation Van Valen put forward what he termed the Red Queen's hypothesis (because of the Lewis Carroll character who found it took all the running one can do to keep in the same place). In concise terms, the hypothesis states that increase in the momentary fitness of any set of species within a specific adaptive zone acts to reduce the fitness of all other species within that zone, in such a way as to cause the effective environment of all the species to deteriorate in a stochastically constant manner through time.

Van Valen's work has aroused considerable interest and provoked active discussion. Foin *et al.* (*Nature*, **257**, 515; 1975) criticise his use of survivorship curves because of the confusion between two quite different methods of analysis and because of the weakness of the analogy between cohorts of organisms born together and taxa which arose at different times. On the other hand Raup (*Paleobiology*, **1**, 82; 1975) generally approves of Van Valen's approach but suggests some methodological improvements; he does not attempt to decide whether or not the proposed law is correct. Another criticism of Foin and his coworkers is that the level of aggregation of the fossil data is so high that it is bound to eliminate all but the most extraordinary events, and apparently log linear survivorship curves are therefore inevitable. To this Van Valen replies that this averaging effect is a positive advantage since it eliminates "noise". He also attempts to clear up a conceptual confusion among his critics. He has never made the sweeping statement that the probability of extinction of a given taxon is independent of age, but argued instead that the mean extinction probability of a group is constant over long periods of time.

Salthe (*Paleobiology*, **1**, 356; 1975) is another who is unimpressed with Van Valen's "law". A stochastic approach to constancy of extinction rates merely signifies that there have been no systematic forces operating on the organisms in question, so that in fact there is nothing that requires explanation in any causal sense. The apparent constancy of extinction has, however, been thrown into question by the methodological analysis of Seposki (*Paleobiology*, **1**, 343; 1975). Seposki points out that the durations of taxa cited by Van Valen are in effect time interval estimates and these time intervals vary in length, approximating to a lognormal distribution. The resultant effect is to impart a systematic bias to the palaeontological data such that linearity, implying constancy of extinction rate, is approximated in the taxonomic survivorship curves. When

corrections are applied, it is estimated that, of Van Valen's 82 survivorship curves for extinct taxa, only 12% are definitely linear. Evidently Van Valen cannot win. Either his results show linearity, which is held to be biologically without significance, or most do not, in which case his "law" breaks down.

Although it begins to look as though the Red Queen has been toppled from her throne, the criticism directed at Van Valen's work should not be held to detract from an original, perceptive and imaginative attempt to glean more from the fossil record than a more conventional approach would allow. It has been stimulating in the best sense and may yet lead to important new advances in our understanding of evolution. That it appeared in the first issue of an obscure journal with limited circulation lends support to the view that if a paper is sufficiently interesting it will be widely read, regardless of where it was published. □

## Strong absorption in $^{40}\text{Ca}$ - $^{40}\text{Ca}$ elastic scattering

from P. E. Hodgson

MANY studies of the elastic scattering of heavy ions have now been made, and it is usually found that the differential cross section has a diffraction structure that varies smoothly with the incident energy and is well described by the optical model.

A notable exception to this behaviour was found some years ago by Bromley *et al.* (*Phys. Rev. Lett.*, **19**, 369; 1967; *Phys. Rev. Lett.*, **20**, 175; 1968): the cross section for  $^{16}\text{O}$ - $^{16}\text{O}$  elastic scattering shows a very irregular behaviour as a function of incident energy. This is in marked contrast to the corresponding data for  $^{18}\text{O}$ - $^{18}\text{O}$ , which varies smoothly with energy as expected.

This early work, together with subsequent studies, showed that this notable difference in behaviour is determined by a balance between the strong absorption of the lower partial waves and the weaker absorption of the higher partial waves. For heavy ion collisions many features of classical behaviour remain, so that partial waves correspond to the interactions at different radial distances: the lower to interactions inside the nucleus and the outer to interactions in the nuclear surface and beyond. The scattering is thus determined by the absorbing part of the optical potential, and it was found that the observed behaviour could well be reproduced by allowing

the strength of the absorption to depend on the orbital angular momentum quantum number  $L$ , being strong for small  $L$  and weak for large  $L$ .

More physically, it was shown that the absorption is mainly due to the direct reactions strongly coupled to the entrance channel. When there are many of these, the nuclear surface is nearly opaque and one observes the small structureless cross section strongly decreasing with energy—behaviour typical of strong absorption. This is the case for  $^{18}\text{O}$ - $^{18}\text{O}$ , which has two neutrons outside the stable  $^{16}\text{O}$  core that can be excited and transferred in many ways, leading to many possible reaction channels.

The situation is quite different for  $^{16}\text{O}$ - $^{16}\text{O}$  which is stable and difficult to excite and so absorbs weakly. Each partial wave makes its own particular contribution, giving the strongly-marked fluctuations in the cross section as a function of energy.

This explanation accounts for all the data in the oxygen region, but it is clearly desirable to confirm it by measurements on other nuclei. It is increasingly difficult to make measurements on heavier nuclei because the strong coulomb repulsion must be overcome before the nuclear interactions can take place, and this requires high incident energies.

The next lightest nucleus after  $^{16}\text{O}$  that has a very stable doubly-closed shell structure is  $^{40}\text{Ca}$ , and so it is desirable to make measurements of  $^{40}\text{Ca}$ - $^{40}\text{Ca}$  scattering. This has recently been done by Doubre *et al.* (*Phys. Rev. Lett.*, **35**, 508; 1975) at Orsay in France, and some of their results are shown in the figure.

It is notable that, contrary to ex-

pectation, the cross sections fall smoothly with increasing energy, so characteristic of strong absorption. An optical model calculation with an absorption increasing with energy agrees well with the experimental data. The physical explanation of this result is not clear. The explanation used in the oxygen region fails for calcium, and it may be that the density of states in the appropriate region of excitation is sufficiently high in the calcium case for the required absorption to occur. More measurements are in progress to provide data for a more comprehensive study of these effects. □

## Dominance and diversity

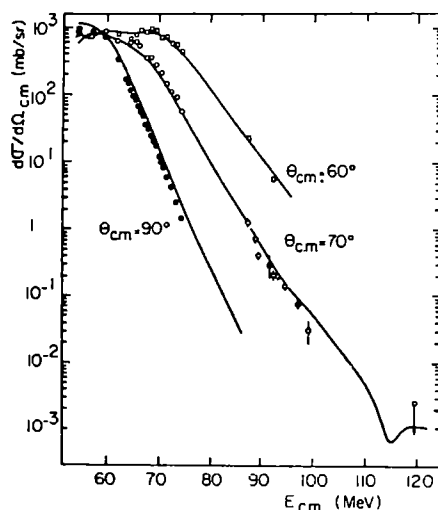
from Peter D. Moore

By far the most frequently used index of diversity in studies of plant communities is that derived from Shannon and Weaver's information theory, in which,

$$H' = -\sum p_i \ln p_i,$$

where  $p_i$  is the proportion of the total number of individuals (or biomass) belonging to the  $i$ th species. This index is a function of both species richness and evenness (the apportionment of individuals or biomass amongst species). The feature of plant communities to which the index responds most effectively is the presence of one or more dominant species. This results both from the consequential inequitable arrangement of biomass or numbers in the community and from the reduction in species number which often accompanies the assertion of dominance by certain members of the community. When one speaks, therefore, of high diversity in plant communities, one is often in fact referring to a lack of dominance.

Because of this it is unwise to assume that diversity changes as a consequence of a particular management practice, such as the abandonment of arable land, or the disturbance of equilibrium communities, can be easily predicted. Such unpredictability is a feature which has been demonstrated in two recent pieces of work in the United States concerned with precisely these situations. Tramer (*Ecology*, **56**, 905; 1975) has studied selected plots on abandoned agricultural land in Ohio over a period of four years and has attempted to document changes in plant species diversity. Numerically based diversity was highest in the second year after abandonment and subsequently fell to a lower level, while biomass-based diversity declined steadily over the four year period. This



Differential cross section for  $^{40}\text{Ca}$ - $^{40}\text{Ca}$  elastic scattering at three angles as a function of energy and compared with optical model calculations.

process can be accounted for by the importance of biennial species in the second year and the subsequent assumption of dominance by the perennials *Aster pilosus* and *Solidago altissima* in the third and fourth year. There is certainly no indication that the popular conception of diversity increasing with succession applies here, but the observations are very short term from a successional point of view.

The dominance of large, perennial plant species in a community must, by its very definition, reduce species diversity. Successional developments involving invasion by such species can therefore be expected to show an accompanying decline in diversity. It is also true that the application of any additional stress, which reduces the performance of a potential dominant, serves to increase diversity, as has been demonstrated by Grime (*Nature*, **242**, 344; 1973) for both man-induced stresses and for natural environmental extremes. One such stress is that of predation, and the experiments of Paine (*Am. Nat.*, **103**, 65; 1966) showed how predation pressures serve to maintain diversity in intertidal communities. The same process operates in terrestrial plant communities grazed by herbivores (see Harper, *Brookhaven Symp. Biol.*, **22**, 48; 1969).

It is this complex interaction of grazing intensity and plant species diversity which provides an explanation for some of the data which Platt (*Ecol. Monogr.*, **45**, 285; 1975) has derived from his study of the recolonisation of areas of prairie disturbed by badgers. He selected for study the abandoned diggings of badgers which had been recovering for at least three years and compared their plant cover with that of the surrounding, undisturbed prairie. In virgin prairie areas, the badger mounds often had more species per unit area, but had a slightly lower overall diversity than their surroundings. This could be related to the important role played by *Andropogon gerardi* on the mounds, which was not observed to the same extent in surrounding areas. Overgrazed prairie regions bore a different plant community in which the grass *Poa pratensis* was dominant, at times accounting for 60% of the above-ground biomass. The diversity of such overgrazed prairie was found to be very much lower than that of virgin areas. Badger mounds in the overgrazed prairie, however, had as high a species diversity as those in virgin areas and were therefore considerably more diverse than surrounding communities. This was the consequence of a reduction in the importance of *Poa pratensis* (to about 20% of the biomass) and also a higher species richness.

Once again the question of successional development is seen to be

irrelevant to the determination of overall plant community diversity, except in that it may relate to the development of dominance. Any factor, whether related to succession or imposed by some external stress, which reduces the establishment or growth rate of potentially dominant, perennial plants, will enhance the species diversity of the community. □

## Hydrothermal activity off-ridge?

from Peter J. Smith

THERE is now considerable evidence to suggest that metalliferous oceanic sediments are generally the result of hydrothermal activity. It is implicit in the close association of most such sediments with submarine volcanism, for example, that the added metals are precipitated from metal-rich solutions produced by the reaction of seawater with cooling magma. In practice, the association with submarine volcanism means that many enriched sediments have been observed in the vicinity of active oceanic ridge crests, for it is there that the bulk of the Earth's volcanic activity occurs. But there are exceptions; metal-rich deposits have been found in, for example, the Bauer Deep. What, then, is the origin of these off-ridge enriched sediments?

As the Bauer Deep lies immediately between the extinct Galapagos Rise and the active East Pacific Rise, it can hardly be said to be totally removed from a ridge environment. One possibility therefore is that the metal-rich deposits (as indicated by high transition metal to aluminium ratios) were formed along the East Pacific Rise by hydrothermal processes and subsequently carried across the rise flank to the Deep. But as Dymond and Veeh (*Earth planet. Sci. Lett.*, **28**, 13; 1975) now point out, there are other possible explanations. There could be a single, wide hydrothermal zone, centred on the East Pacific Rise and encompassing the Bauer Deep, within which the metal/aluminium deposition varies. Alternatively, the Rise and the Deep could be associated with separate hydrothermal systems. Or the Rise crest sediments could be hydrothermal and the Deep sediments largely authigenic.

In an attempt to determine which, if any, of these models is the correct one, Dymond and Veeh have analysed metal accumulation rates in cores from the East Pacific Rise crest, the flanks of the Rise and the Bauer Deep. The role of aluminium in marine environments is poorly understood, and so the significance of variations in the transition metal/aluminium ratio itself is difficult to assess. Nevertheless, the separate

patterns of accumulation rates of Fe, Mn and Al across the Rise-Deep zone seem to be inconsistent with the patterns expected from a wide hydrothermal region. The observed patterns all peak in the Deep as well as at the Rise, suggesting that the idea of a slow decrease in hydrothermal activity from the Rise outwards cannot be valid.

The peaking across the Deep also rules out a more rapid fall of hydrothermal activity away from the Rise in conjunction with completely authigenic precipitation in the vicinity of the Deep. Estimates made by Dymond and Veeh suggest that 30-50% of the manganese and all of the nickel in the Bauer Deep could in fact be deposited by authigenic processes; and given the errors on these estimates, this means that an authigenic origin for most of the minor transition metals cannot be ruled out. But the same cannot be said for iron. The amount of iron present is such as to eliminate authigenic activity as a primary source, even if it is allowed that some of the iron could be detritus from land-derived clays and volcanic debris.

This then leaves two models: the double (Rise and Deep) hydrothermal source and the single (Rise) source with transport of metal-rich sediment to the Deep. Unfortunately, since the two models give similar distributions of metals, it is not possible to distinguish between them on the basis of Fe and Al accumulation rate data alone. Although there is a maximum in metal accumulation over the Deep, there is apparently no comparable maximum on the opposite side of the Rise. This would seem to argue against a single source with transport, except that preferential transport of material away from the Rise in one direction cannot be ruled out (especially as knowledge of the bottom currents in the area is poor).

Then again, Anderson and Halunen (*Nature*, **251**, 473; 1974) have argued in favour of hydrothermal processes in the Bauer Deep on the basis of heat flow data, suggesting that such processes could have been initiated when spreading moved from the Galapagos Rise to the East Pacific Rise about 6.5 Myr ago. On the other hand, one of the Deep cores examined by Dymond and Veeh is probably about 20 Myr old; and persistent hydrothermal activity since this core was formed seems unlikely.

There is thus evidence (that given above and more) on both sides. Moreover, there are data suggesting that neither model is entirely correct. There are, for example, chemical differences between East Pacific Rise and Bauer Deep sediments, although many, if not all, of these could be explained on the basis of simultaneous authigenic depo-

sition in the Deep. For the time being the detailed picture remains unclear. What is certain, however, is that the rate of accumulation of metals in the Bauer Deep is an order of magnitude lower than that on the East Pacific Rise. □

## "Turn-coat" trypanosomes undressed

from J. R. Baker

It has been known for some time that the tsetse-transmitted trypanosomes of Africa (including *Trypanosoma brucei*, which causes human sleeping sickness) have, at certain times in their life cycle, a 12–15 nm thick "coat" outside their unit plasma membrane. Mainly circumstantial evidence strongly suggested that this coat contained antigens presented to the vertebrate host which, by being structurally changed every time the host produced a specific antibody, contributed to the parasites' survival and, hence, the eventual death of the host (Vickerman, *Ciba Symposium*, **25**, 53; 1974) and also to our inability to produce an effective anti-trypanosomiasis vaccine.

A few years ago, Wright and Hales in Toronto (*J. Parasit.*, **56**, 671; 1970) suggested that the coat consisted of carbohydrate and protein, and work by them and Lumsden (*J. Cell Sci.*, **6**, 285; 1970) led to the idea that the trypanosomes may cast off old antigen by releasing long tubular threads of encoated plasma membrane (though the natural production of these threads *in vivo* has never been conclusively demonstrated). In Basel, Steiger produced evidence that new coat was produced by the trypanosomes' Golgi-derived reticulum (*Acta trop.*, **28**, 341; 1971 and **30**, 64; 1973). This study took an exciting step forward three years ago when Cross reported to the 13th Trypanosomiasis Seminar in London the isolation of a single glycoprotein apparently representing dissociated coat (*Trans. R. Soc. trop. Med. Hyg.*, **67**, 261; 1973). Significantly, the putative coat proteins prepared from different clones of one strain of *T. brucei* differed in composition. This supported the view that coat protein is the variant antigen, an idea now further strengthened by Cross's latest discovery that mice immunised with purified coat glycoprotein (molecular weight  $65,000 \pm 3,000$ ) are protected against subsequent infection with living trypanosomes of the same, but not of other, clones (*Parasitology*, **71**, 393; 1975). One of the major remaining problems concerns the cellular control of this antigen switch; it is insufficiently random to be explained easily by mutation and its occurrence within a

clone appears to preclude its resulting by selection from a pre-existent mixed population. Current thinking—stemming, incidentally, from work by Ehrlich and others in 1909 (*Z. Immunitätsforsch.*, **3**, 296; 1909)—favours a rather vague concept of induction by antibody. Cross's work may well prove to be an important key in the eventual unlocking of this mystery which, in turn, could conceivably lead to successful vaccination against sleeping sickness. □



## A hundred years ago

A REMARKABLY valuable discussion by M Belgrand, of the inundations of the Garonne, viewed specially in connection with the heavy rains which fell over France from the 21st to the 24th of June last, has been appearing at intervals for the past fortnight in the *Bulletin International* of the Paris Observatory. It is pointed out, from the dates of their occurrence, that the inundations of the southern portion of the basin of the Garonne which slants from the Pyrénées, have nearly always occurred in spring or early summer, and at the same dates either no floods at all, or comparatively unimportant floods, were experienced in the northern portion of the basin which slopes down from the Cevennes and central plateaux of France. It is to be noted that it is just at this season that the rainfall of the southern portion of France attains its annual maximum, and the nearer to the Pyrénées the more decidedly is the May–June maximum marked, and that the melting of the snows which have accumulated on the Pyrénées during the winter months proceeds most rapidly. On the other hand, it is shown that the great inundations of the northern portion of the basin occur generally during the cold months of the year, and that at the time of their occurrence there have been no corresponding great floods at Toulouse, in the southern portion of the basin. It is during the cold season that the rainfall reaches its annual maximum on leaving the slopes of the Pyrénées and advancing northwards over the basins of the Tarn, Lot, and Dordogne. The disastrous inundation of June, 1875, was in accordance with the experience of previous floods in the south of France. As a great flood it was limited to the river courses sloping down from the Pyrénées; and the nearest approach to a great flood elsewhere was in the basin of the Argout, the most southern tributary of the Tarn, and it was the flood of this tributary which occasioned almost the whole of the flood of the Tarn. At such places as Auch, situated in a narrow valley, and where, consequently, the drainage area is small, the inundation was much less disastrous than at Toulouse and places similarly situated near the confluence of large affluents draining a wide extent of the country.

from *Nature*, **13**, January 6, 197, 1876

## Oscillating universe bounces back

from John Gribbin

THE biggest problem with the Big Bang theory of the origin of the Universe is philosophical—perhaps even theological—what was there before the bang? This problem alone was sufficient to give a great initial impetus to the Steady State theory; but with that theory now sadly in conflict with the observations, the best way round this initial difficulty is provided by a model in which the Universe expands from a singularity, collapses back again, and repeats the cycle indefinitely. Such models have been known since the earliest development of relativistic cosmologies in this century, but have been favoured with a revival of interest in recent years. The latest study provides some intriguing agreements with observation of the real Universe, but also poses some new puzzles (Landsberg and Park, *Proc. R. Soc. Lond.*, **A346**, 485–495; 1975).

Landsberg and Park consider a model universe composed of gas and radiation which are 'allowed to interact'; this incorporates features of standard cosmology (taking account of gravitation) and of statistical mechanics (which does not take account of gravitation). The resulting hybrid is, they suggest "too simple to be realistic", but does go a step further than the standard dynamic models of traditional cosmology. Both the gas and the radiation (assumed black body) are uniform, which is as good an approximation as in any other cosmology, where such incidentals as galaxies are generally taken as 'test particles' of no great account.

The first important result which emerges from this incorporation of thermodynamics into cosmology is that during both the expanding and contracting phases of the oscillating model entropy increases, so that the "arrow of time" is constant. To follow the repeated 'bounce' through the initial/final singularity Landsberg and Park cheat a little by simply reversing the collapse at very great density, so that it becomes an expansion with the same speed. This may not be entirely valid, since it carries over information from one cycle to the next, but because the collapse phase proceeds more rapidly than the preceding expansion, this means that successive cycles of the oscillation start out faster and faster, expanding to greater and greater radii before they collapse in their turn, with each cycle taking longer than the last. This behaviour is in line with that of earlier oscillating models, but even

though it always happens with the models they have calculated, Landsberg and Park cannot prove that it must happen.

Such behaviour could explain why our Universe seems to be so close to the dividing line between continued infinite expansion and just turning back into collapse at some future time. In the Landsberg-Park cosmology, successive cycles of expansion/collapse bring the model ever closer to this dividing line, and the closeness of our Universe to the "just bound" condition might suggest that it has already been through many cycles. So, like the best theories, this one makes a firm prediction: future observations should show that the Universe is indeed just bound, and not just unbound.

But there are two snags with the theory. First, if successive cycles expand to bigger and bigger radii, running the calculation backwards would suggest that the bounce itself started in some infinitesimal hiccup some large number of cycles ago, so we still have an origin problem but in a different form. Second, and perhaps more serious, if the closeness of the Universe to the "just bound" condition means that it has been through many cycles then there has been ample opportunity for entropy to increase following the constant thermodynamic arrow of time of the model. Yet the most obvious feature of our Universe is its low entropy; as Hermann Bondi has said "thermodynamic properties tend to be very deep and significant: the fact that our night sky is very black, with very bright points, the stars, in it, may be the profoundest piece of knowledge of the universe that we have". But these, of course, are just the kind of questions that a more sophisticated version of this simple model might be expected to tackle. □

## Rotation of planetary atmospheres

*from a Correspondent*

A meeting of the Royal Astronomical Society, Institute of Physics and the Royal Meteorological Society, held in London on November 28, 1975, provided interdisciplinary discussions of the atmospheric motions at all atmospheric levels.

STUDIES of atmospheric motions concern scientists from various backgrounds, whose research interests generally confine them to a small region of the entire atmosphere. Meteorologists, for example, are concerned with

the lowest levels which constitute the troposphere and stratosphere which extend from the surface to about 35 km for their studies of weather and climate; aeronomers are interested in the physics, chemistry and motions of the remaining portions of the neutral atmosphere; the stratosphere, mesosphere and thermosphere, which extend to about 100 km above the surface; while a third group study the ionised layers of the upper atmosphere. It may be scientifically convenient to partition the atmosphere in this way according to the dominant physical processes that occur. It is, however, of fundamental importance that we now study the whole atmosphere, and consider the energy transfers between these regions, so that we may determine whether external effects such as solar activity or stratospheric compositional changes could have any effect upon our climate in the troposphere.

By tracking orbiting satellites for more than a decade, D. King-Hele (Royal Aircraft Establishment, Farnborough) has found that Earth's atmosphere above about 100 km moves faster than the solid body of the planet. This is called "super rotation". The magnitude of the motion is highly variable, with altitude, latitude and time, although the largest value measured so far is about  $160 \text{ m s}^{-1}$  at an altitude of 240 km from tracking the COSMOS 344 satellite. The normal rotation at these levels would imply motions of  $100 \text{ m s}^{-1}$ . The Explorer 1 satellite has been tracked through two solar maxima, and suggested that the rotation rate decreased with a corresponding decrease in solar activity. Subrotational motions ( $\leq 100 \text{ m s}^{-1}$ ) have so far been found only in the afternoon period at these altitudes. H. Rishbeth (SRC Appleton Laboratory, Slough) indicated that there is inadequate information to construct a theoretical model in an attempt to explain these motions. Unlike the winds in the lower atmosphere, they were not in geostrophic balance at these levels. He indicated that the driving mechanisms would differ on a local basis (for example, the polar cap, auroral zone, equatorial latitudes) and an integrated measurement/theoretical programme was urgently required. The deposition of interplanetary matter, in particular meteoroids, has often been thought to be a possible additional heating source of the upper atmosphere. D. W. Hughes (University of Sheffield) showed however, that there is insufficient material to modify the upper atmospheric motions in any significant manner.

In the stratosphere, wave motions play a major role in modifying the atmospheric motions as discussed by

D. G. Andrews (University College, London; Meteorological Office). The equatorial region has been found to undergo a quasi-biennial oscillation (QBO) in which, in a period of about 26 months, easterly motions become reversed into westerly motions. He suggested that Kelvin waves with a 15-d period could account for the easterly flow, while a Rossby gravity wave with a 4-d period could be responsible for the westerly flow. Andrews also thought that tropospheric changes may influence these stratospheric motions through the vertical propagation of energy. A. White (Imperial College, London) discussed the importance of baroclinic eddies in the transfer of troposphere energy. He showed that the structure of the flow was sensitive to the pole-to-equator temperature gradient and that changes in this parameter would modify the flow, producing a different climatology. White's studies indicated that simple models may be constructed to study climatic change over time intervals of several decades, through the parameterisation of the heat transfer effects of these eddies.

Other planetary atmospheres exhibit super rotation features, whose understanding could assist in determining the dominant driving mechanisms of the corresponding features of the Earth's atmosphere. R. A. Plumb (Meteorological Office) discussed the rotation of the Venusian stratosphere which moves sixty times faster than the solid body of the planet, producing zonal winds of about  $100 \text{ m s}^{-1}$ . The motion is generated by the upward momentum transport by the thermal tide in the Venusian stratosphere, driven by solar energy absorbed by the opaque clouds whose tops occur at about 200 mbar. Similar thermal forcing could not occur in the Earth's atmosphere, since most of the solar radiation is absorbed at the surface. Jupiter and Saturn also exhibit equatorial jets with zonal velocities of about 100 and  $400 \text{ m s}^{-1}$  respectively. R. Hide (Meteorological Office) stated that currently available theories were unable to account for these features.

This meeting emphasised the importance of the transfer of energy between atmospheric regions in explaining many of the observed motions. A more detailed understanding of the atmospheric dynamics requires further observations at all levels, which for the Earth's atmosphere is possible through the presently planned satellite and rocket programme as D. Rees (University College London) described. We may then expect further tripartite meetings of this type which are an invaluable forum for discussing the motions of planetary atmospheres. □



# articles

## High resolution observations of NGC1275 with a four-element intercontinental radio interferometer

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*The radio nucleus of NGC1275 (3C84) is found to consist of three apparently stationary centres of emission extending over 0.006" (3 pc) along position angle  $-9^\circ$ . One or more of these centres have changed their intensity or size in the last 3 yr. A preferential direction of alignment of both the small scale and large scale radio features as well as the optical features suggests a common cause of alignment operating over linear distances from a few pc to  $> 100$  kpc.*

NGC1275 is an active radio galaxy and is one of the strongest extragalactic radio sources at centimetre wavelengths. The radio nucleus has been previously studied by very long baseline interferometer techniques<sup>1-5</sup>, and has been shown to have a complex angular structure  $\sim 0.006''$  in extent and elongated along position angle  $\sim -10^\circ$ . Because the radio nucleus has a relatively large angular size and a high brightness, it is uniquely suited for detailed high-resolution observations. The earlier measurements, however, had an insufficient number of antenna pairs and insufficient resolution to map the complex structure in any detail.

### Intercontinental interferometer system

We report here new observations made at epoch 1974.5 with a 4-element intercontinental interferometer system which gave six simultaneous independent interferometers. The

observing frequency was 10,650 MHz ( $\lambda = 2.8$  cm) giving a maximum baseline of  $290 \times 10^6 \lambda$ . This was sufficient to resolve a Gaussian source of  $0.00025''$ . The polarisation was left-circular.

The elements of the interferometer are described in Table 1 and the diurnal track of each interferometer in the Fourier transform ( $u, v$ ) plane is illustrated in Fig. 1. Data were recorded at each station on an NRAO Mk II VLB terminal, and the tapes were correlated on the NRAO processor<sup>6</sup>. A description of the further averaging of the data, the calibration of the fringe amplitude and the experimental errors has been given elsewhere<sup>7</sup>.

### Derivation of source structure

The observed data are shown in Fig. 2 in the form of plots of the fringe amplitude as a function of the interferometer hour angle. (The interferometer hour angle (IHA) is defined as the hour angle of the source measured from the meridian of the equatorial projection of the baseline.) The location in the ( $u, v$ ) plane of each of the maxima and minima in the visibility function is indicated in Fig. 1.

The separation and direction of lines joining corresponding maxima and minima of the visibility function demonstrate the multiplicity of components and give the approximate extent of the source and the direction of elongation. In the inner part of the ( $u, v$ ) plane the spacing corresponds to a total separation of about  $0.006''$  between the outer major features; the somewhat closer spacing of the extrema on the longer baselines requires weak compact features separated by up to  $0.01''$ . The

Table 1 Interferometer elements

Location*	Diameter (m)	System noise temperature (K)	Frequency standard	Institution
Effelsberg, W. Germany	100	300	Rb	MPiR
Green Bank, W. Virginia, USA	43	120	Maser	NRAO
Fort Davis, Texas, USA	26	300	Rb**	HRAS
Big Pine, California, USA	40	225	Rb**	OVRO

\*The geographic location of the stations and the baseline lengths have been given in ref. 7.

\*\*A quartz oscillator was used to reduce the short term noise from the rubidium standard.

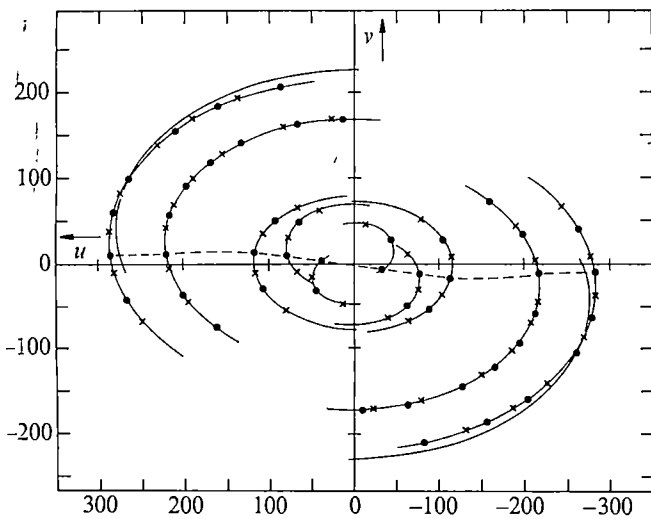


Fig. 1 Diurnal tracks for the six interferometers in the  $(u, v)$  plane. The units in  $u$  and  $v$  are in  $\lambda \times 10^6$ . Maxima and minima in the fringe visibility are shown by filled circles and crosses, respectively, except on one track (MPIR-HRAS), where the data are sparse. The line joining the principal maxima is shown dashed.

decrease in fringe amplitude along the line of the principal maximum (the line through the origin) shows that the size of the source along the minor axis is about  $0.0015''$ . As can be seen from Fig. 2, the maximum fringe amplitude on the three longer baselines is only  $\sim 10\%$  of the total flux density of  $58.4$  Jy, while on the shortest baseline the fringe visibility reaches  $90\%$ . Most of the emission therefore originates in components larger than  $0.001''$ . The rapid changes in fringe amplitude with hour angle observed on the trans-Atlantic baselines are real and the data have been confirmed by repeated observations.

A model describing the source brightness distribution was derived as follows: first, the overall extent and elongation of the source was determined by inspection of Fig. 2. Next, the brightness distribution was approximated by a model consisting of 10 elliptical Gaussian components. The parameters of each of these components were found by an iterative procedure which minimised the r.m.s. deviations between the observed data and the visibility function of the model. This preliminary model was then decomposed into a grid of point sources spaced  $0.0002''$  apart. The amplitude at each grid point was varied by another iterative procedure which further reduced the r.m.s. deviations between the observed visibility function and that of the model. Finally, the resulting grid was smoothed with a Gaussian beam having a FWHM of  $0.0004''$ . The resulting map of the radio structure of the NGC1275 nucleus is shown in Fig. 3, and the calculated fringe amplitudes of the unsmoothed model as a function of the IHA are shown in Fig. 2, together with the experimental data.

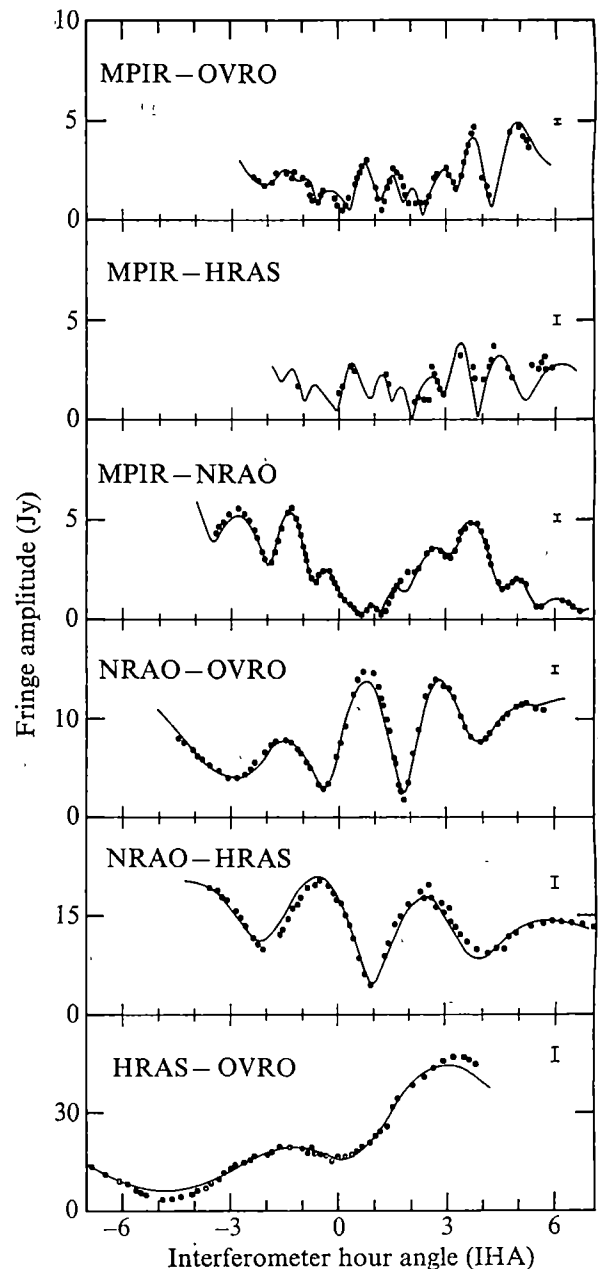
We emphasise that the map shown in Fig. 3 is based on fringe amplitudes alone, since we did not retain the phase information necessary to perform a Fourier inversion of the data. Models derived in this way are not unique; in particular, there is an ambiguity in the position angle of  $180^\circ$ . Also, the coverage of the  $(u, v)$  plane is incomplete, and even with phase information this would have led to sidelobes in the reconstructed map. To investigate the uniqueness of our model, we have used the iterative grid procedure starting from six-component and two-component models, rather than the ten components used to produce the map shown in Fig. 3. Each of these approaches gave very similar final maps. Moreover, four of the authors have independently derived a map using multi-component Gaussian models and different modelling procedures, and again these maps agree in their main features.

This is perhaps not surprising since the complexity of the

fringe visibility function, coupled with the restriction that the brightness should everywhere be positive, seems to provide constraints which facilitate the reconstruction of the main features of the brightness distribution even in the absence of phase data. It is always possible to find a more complex geometry which gives an even better fit, but the data do not justify more complex models. We believe, however, that the data do require a model of the complexity of that shown in Fig. 3. We note that the evidence for fine structure on a scale  $\lesssim 0.001''$  comes primarily from the trans-Atlantic baselines. A much simpler model, consisting of five Gaussian components, fits the data from the three shortest baselines adequately, and reproduces the trend of the data from the longer baselines with deviations of only a few Jy.

As a further comparison, we have convolved our map with

Fig. 2 The fringe amplitude as a function of IHA for the six interferometers. The experimental data are shown as filled circles. The experimental errors for each interferometer are indicated by error bars at the right hand side of each plot. These are the errors corresponding to the largest fringe amplitude observed; errors near the minima of the fringe visibility are smaller than the size of the plotted symbols. The computed fringe amplitudes for the model structure are shown as solid curves.



a fan beam to produce a one-dimensional strip scan along the major axis. We find that this agrees qualitatively with the one-dimensional brightness distribution which has been derived<sup>3</sup> from amplitude and phase-closure data obtained at 3.8 cm at epoch 1973.7.

### Nature of radio source

The major features of the radio nucleus of NGC1275 seen in Fig. 3 are the three areas of high brightness. The separation between the peaks of the two outer regions is  $0.006''$  and the overall extent along the major axis down to the 10% contour level is  $0.01''$ . The centres of the three major peaks lie nearly on a straight line in position angle  $-9^\circ$ . In this sense the structure is 'colinear' although there are clearly significant deviations from a simple linear structure. The size (FWHM) along the minor axis is  $\sim 0.0015''$ . The brightest feature is located near the centre of the source and has a peak brightness temperature of about  $10^{11}$  K on the smoothed map. Observations of still higher resolution will be required to study this compact region.

The redshift of NGC1275 is 0.018, corresponding to a distance of 110 Mpc ( $H = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ ), so that  $0.001''$  is equivalent to a linear distance of 0.5 pc. The separation of the two major outer components is then 3 pc, and a significant part of the emission from the central component originates from a region smaller than 0.1 pc.

In Fig. 4 we have plotted the radio-frequency spectrum of the source 3C84 between 20 MHz and 100 GHz using the data taken from the literature as indicated. The spectrum can be broken down into three parts<sup>8,9</sup>, associated with structure on different angular scales:

(A) A high frequency spectral component associated with the radio structure discussed here. This component becomes self-absorbed below about 5 GHz. At 10.65 GHz the measured total flux density was 58.4 Jy. Extrapolation of the two components B and C indicates that they contribute about 1.5 Jy at 10.65 GHz and our model of component A accounts for all the remaining flux density.

(B) A decimetre wavelength spectral component which becomes self-absorbed below 500 MHz. Purcell (personal communication) has studied this component with the NRAO-HRAS-OVRO interferometer at 609 MHz. The structure is elongated at a position angle near  $0^\circ$ , and is probably double with a component separation of about  $0.05''$  (25 pc). The available data do not allow the relative positions of component A and the double source making up component B to be determined accurately. The centroids of the two components must, however, lie within a few arc seconds of each other.

(C) A component with a 'normal' power-law spectrum. This extended feature has been studied<sup>15</sup> with the Westerbork array at 1,420 MHz. It consists of a  $30''$  (15 kpc) source coincident with the galaxy and a larger,  $5'$  source (150 kpc), both of which are elongated roughly along position angle  $-10^\circ$ .

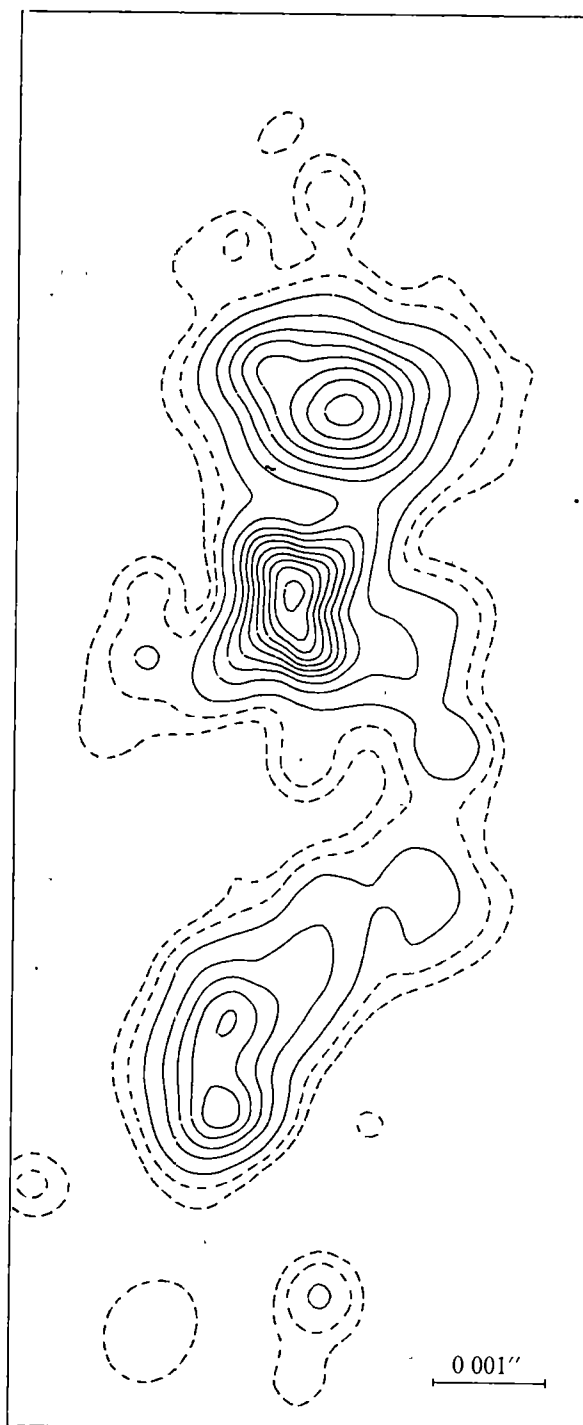
Each of the components A, B, and C is elongated in essentially the same direction. Moreover, the H $\alpha$  filaments, which extend<sup>16</sup> more than  $140''$  from the centre of the galaxy, also have their greatest extent in this direction. This suggests a common mechanism of alignment which is effective over linear scales ranging from a few pc in the dense nucleus of the galaxy to more than 100 kpc in intergalactic space.

The 150 kpc component must be at least  $5 \times 10^5$  yr old and is probably 10–100 times older. Component A, on the other hand, is probably not more than 20 yr old. Although the earliest observations<sup>17</sup> of the flux density of 3C84 at 10.7 GHz were made in 1966, measurements at 8 GHz (ref. 18) go back to 1960 at which time the flux density was only about 10 Jy. At the time of our measurements, however, the flux density at 8 GHz had increased to 55 Jy (Blankenship, personal communication), so that most of the present component A did not exist before 1960. Numerous observations at centimetre wavelengths since 1965 show that the flux density of component A increased in an approximately linear manner until

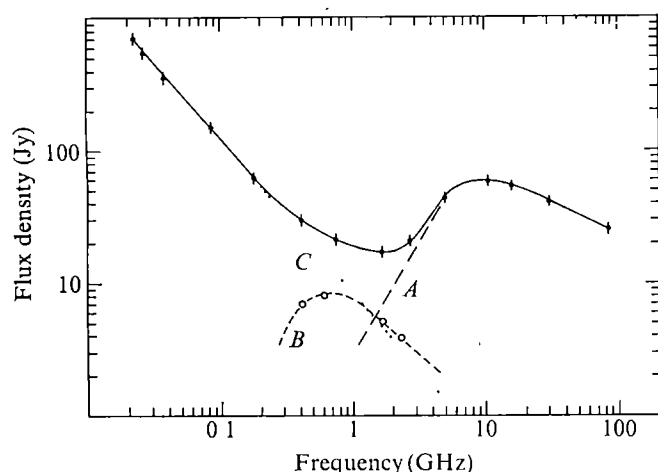
1973 and has remained roughly constant since that time (for example, refs 5, 19, 20).

Unfortunately, we have high resolution observations beginning only in 1972, so that we cannot trace the development of component A in detail. Previous interferometer data<sup>5</sup> taken at 10.7 GHz with an angular resolution of about  $0.001''$ , made with the NRAO-HRAS-OVRO antennae at the epochs 1972 April, 1972 October and 1973 March, show that the maxima and minima in the fringe visibility have remained at nearly constant positions in the  $(u,v)$  plane, while the amplitudes of the extrema have varied considerably over this time.

Fig. 3 The structure of the radio nucleus of NGC1275, derived as described in the text. Contours are given in 10% intervals of the maximum brightness down to the 10% level; the 5 and 2.5% levels are shown dashed. The scale in the two coordinates is indicated by the  $0.001''$  bar in the lower right corner. North is at the top and East at the left, but there is a  $180^\circ$  ambiguity in the orientation of the map (see text)



This implies that the overall dimensions of the source and the relative locations of the three bright condensations have not changed significantly, but their intensity and/or size has varied during this time. Thus the nucleus of NGC1275 seems to contain three apparently stationary centres, separated by



**Fig. 4** The radio spectrum of NGC1275. Above 1 GHz, the epoch is that of the present measurements (1974.5). Total flux density data below 1.65 GHz are taken from Ryle and Windram (ref. 8); at 1.65 GHz from unpublished data obtained by Webber, Denoyer and Swenson; above this frequency from unpublished measurements made by the authors and A. Niell. The separation into spectral components A (—), B (---) and C (· · ·) is based on interferometer data at 408 MHz and 1.4 GHz (ref. 10); 448 MHz (ref. 11); 609 MHz (Purcell, personal communication); 1.4, 2.7 and 5 GHz (ref. 12); 1.65 GHz (ref. 13) and 2.3 GHz (ref. 14, and unpublished data). In making the separation, we have assumed that components B and C have not varied during the period 1969 to 1974.

a few pc and lying nearly along a straight line; at least one of these centres has been active in the past three years. This is in contrast with the quasar 3C273 and the radio galaxy 3C120, where the location of the extrema changes rapidly<sup>6</sup>, suggesting rapid movements of the radiating components.

Repeated observations with multi-element intercontinental interferometer systems are necessary to study the details of the time variations in the nucleus of NGC1275. This would give unprecedented insight into the powerful energy source located in the nuclei of galaxies and specifically into the mechanism responsible for the origin of extragalactic radio sources.

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# The structure of the protein disk of tobacco mosaic virus to 5 Å resolution

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*An electron density map of the TMV disk at 5 Å resolution has been obtained using isomorphous replacement and non-crystallographic symmetry. The polypeptide chain can be traced with little ambiguity. The axial contacts between protein subunits are unlike those in the virus, the disk being a more open structure apparently designed for rapid interaction with the RNA.*

THE disk of tobacco mosaic virus (TMV) protein is crucial in the assembly of the virus from its RNA and protein. It is composed of two rings each containing 17 protein subunits<sup>1</sup>, both layers facing the same way<sup>2</sup>. The disk has long been known as one of the polymorphic forms of TMV protein<sup>3</sup>, but it was only recently shown to be the dominant aggregate at neutral pH and moderate ionic strength<sup>4</sup>, essential for nucleation of the virus assembly, and to contribute to the rapid elongation of the nucleoprotein rod<sup>5,6</sup>. The interaction of the disk with a special sequence on the RNA also provides a mechanism for recognition by the protein of its homologous RNA<sup>5</sup>.

The X-ray diffraction study of the disk was begun before its relevance to virus assembly had been established, when crystals were obtained during systematic attempts to crystallise small aggregates of the subunits<sup>1</sup>. These earlier X-ray studies, leading to a low resolution electron density map, have been described<sup>7–9</sup>. Because of the large size of the unit cell, which has a whole disk of molecular weight 600,000 as its asymmetric unit, the extension to high resolution posed a formidable technical problem, which was overcome only by the development in this laboratory of an automated camera, together with a computer-linked densitometer and associated computer programs for processing the hundreds of thousands of X-ray intensities involved<sup>10–12</sup>. Here we describe the extension of the X-ray work to 5 Å resolution.

The structure has been solved by the method of isomorphous replacement. Only two heavy-atom derivatives were used, but good phases were nevertheless obtained by using the 17-fold rotational symmetry of the disk; this non-crystallographic axis gives rise to redundant information in the X-ray data which can be exploited in phase determination. The validity of the final electron density map is demonstrated by the striking similarity between the two



rings of the disk, which are crystallographically independent and whose similarity was not assumed at any stage. The map is sufficiently clear for the course of the polypeptide chain to be traced with little ambiguity.

### X-ray data collection

The preparation and crystallisation of the virus protein has been described by Leberman *et al.*<sup>7</sup>. The crystals are orthorhombic, space group  $P2_22_1$ , with unit cell  $a=228$  Å,  $b=224$  Å and  $c=174$  Å. The disks stack in pairs related by the crystal dyad<sup>8</sup> with their 17-fold rotation axis approximately parallel to  $c$ .

There are 39,000 unique reflections to 5 Å, and a complete set of data, allowing for scaling overlaps, requires the recording of about 200,000 reflections. X-ray intensities were first collected from crystals of the native protein and of a covalently-bound methylmercury derivative<sup>7</sup>. Subsequently, to check and improve the phasing, data were also collected from a  $(AuCl)_2^-$  derivative, but this proved to have low occupancy.

X-ray photographs were taken on an Arndt-Wonacott oscillation camera<sup>10</sup> using small contiguous rotations, about the  $b$  axis, of approximately  $1^\circ$  per film pack. The total rotation angle required was  $90^\circ$ , necessitating several crystals for each set of data. Reflections occurring at the

Table 1 Statistics of data collection to 5 Å

	Native	Mercury	Gold
Total reflections measured	181,000	198,000	118,000
No. of split reflections	57,000	73,000	42,000
No. of independent reflections	38,800	39,100	37,600
$R_{\text{sym}} = \sum_i  \bar{F}_i - F_i  / \sum_i \bar{F}_i$	0.14	0.10	0.13
$R_{\text{sym}}$ , 50% strongest reflections	0.08	0.06	0.07
Mean isomorphous difference $\langle  F_{\text{nat}} - F_{\text{der}}  \rangle / \langle F_{\text{der}} \rangle$	—	0.30	0.19

The films were scaled together by means of symmetry-related reflections recorded on different films and a symmetry  $R$  factor was computed for each compiled set of data. Split reflections could not be used for scaling but were included in the statistics. Their exclusion did not significantly improve the  $R$  factor.

boundaries of contiguous rotations were split between consecutive films, and such data were assimilated by adding the integrated intensities of the separate parts from each film<sup>10</sup>. Films were measured on a flat-bed scanner (J. F. W. Mallett, T. H. Gossling, A. R. Faruqi, and J.N.C., unpublished) which combines the positional accuracy of a mechanical stage<sup>11</sup> with the speed of a flying-spot densitometer<sup>12</sup>. The data quality (Table 1) is poor by small protein standards because of the high proportion of weak reflections and the limited exposures, although these were already several hours per degree of oscillation on a rotating-anode X-ray tube. We accepted this lower standard, however, because of the expectation, which was later justified, that the 17-fold redundancy implicit in the data would compensate for this. Better data were obtained for the mercury derivative, for which larger crystals and longer exposures were used.

### Phase determination

Phases were obtained by a combination<sup>13</sup> of isomorphous replacement and non-crystallographic symmetry<sup>13,14</sup>. The mercury positions found by Gilbert and Klug<sup>9</sup> from the centrosymmetric zones were refined using 20,000 selected reflections. The refined positions were used both to locate the non-crystallographic 17-fold axis precisely and to calculate a single isomorphous map. The asymmetric unit was isolated, averaged 17-fold, and the averaged structure replaced in the unit cell. The averaged map was then used to compute a set of structure factors ( $F_{\text{calc}}$ ). Treating the map

Table 2 Progress of the phase determinations starting from single (SIR) and double (DIR) isomorphous replacement

	SIR			DIR			
Cycle	0	1	2	0	1	2	3
$R$ factor after averaging		0.38	0.35		0.39	0.31	0.28
Figure of merit	0.48	0.67	0.68	0.60	0.74	0.77	0.78
Mean phase change from last cycle		34.5°	3.4°		24.7°	6.5°	2.9°

$R = \langle |F_{\text{obs}} - F_{\text{calc}}| \rangle / \langle F_{\text{obs}} \rangle$ , where  $F_{\text{calc}}$  is the structure factor computed from the average map.

as the known part of a total structure, phase-probability distributions were computed from the  $F_{\text{calc}}$  using Sim's formula<sup>15</sup>. The sharpness of the distributions depends on the agreement between the measured amplitudes ( $F_{\text{obs}}$ ) and the  $F_{\text{calc}}$  over the complete set of data. These distributions were combined multiplicatively with those obtained from isomorphous replacement to calculate new best phases and figures of merit. Attaching these to the  $F_{\text{obs}}$ , another map was calculated, enabling the cycle of averaging and phase-recombination to be repeated.

After only two or three cycles, the mean phase change per cycle was as little as  $3^\circ$ . Phases from the final cycle were used to locate the heavy atoms in the gold derivative, and ultimately the above procedure was repeated starting from double isomorphous phases. The course of each phase determination is summarised in Table 2. The final mean figure of merit was higher for the determination starting with the double isomorphous phases (0.78) than for that starting with the single (0.68). Nevertheless, the final maps in each case were of comparable quality, showing that the use of the non-crystallographic symmetry was of greater value than the second isomorphous derivative. The heavy-atom parameters for the two derivatives are given in Table 3. The hand was determined from the anomalous differences in the derivative data.

### The electron density map and its interpretation

The map in Fig. 1 is the 17-fold averaged structure and is plotted on sections perpendicular to the 17-fold axis. The figure shows approximately  $4\pi/17$  rad of the disk (two subunits wide) sliced into four thick composite sections, A1, A2, B1 and B2, each about half a layer deep. The two layers are denoted A and B, where A is closer to the crystal dyad, and each ring is divided into two parts, 1 and 2, to give the four sections (Fig. 2). Each layer contains two slewed rods of density (LS, RS) per subunit in the upper half and two radially oriented rods (LR, RR) underneath

Table 3 Heavy atom parameters

	Mercury		Gold	
	Ring A	Ring B	Ring A	Ring B
Relative occupancy	58	58	23	14
Radius in Å	59.2	58.9	72.5	70.1
Height above dyad in Å	12.1	38.8	3.5	28.9
Azimuth ( $\phi$ )	4.1°	8.0°	-0.6°	3.2°
Phasing power $\langle f_H \text{ calc} \rangle / \langle E \rangle$	2.78		1.10	

$f_H \text{ calc}$ , Calculated heavy atom structure factor.

$E$ , lack of closure error.

The 34 sites for each derivative were refined against the phases calculated by combining the probability distributions for the DIR phases (in which the weight of the gold contribution had been doubled) with those resulting from two cycles of averaging and recombination of the SIR phases. This procedure was necessary to prevent the mercury derivatives from completely dominating the DIR phase calculation and refinement. The Table gives the 17-fold averages of the parameters refined for individual atoms. None of the mercury positions differed by more than 0.3 Å from those corresponding to perfect symmetry. R385

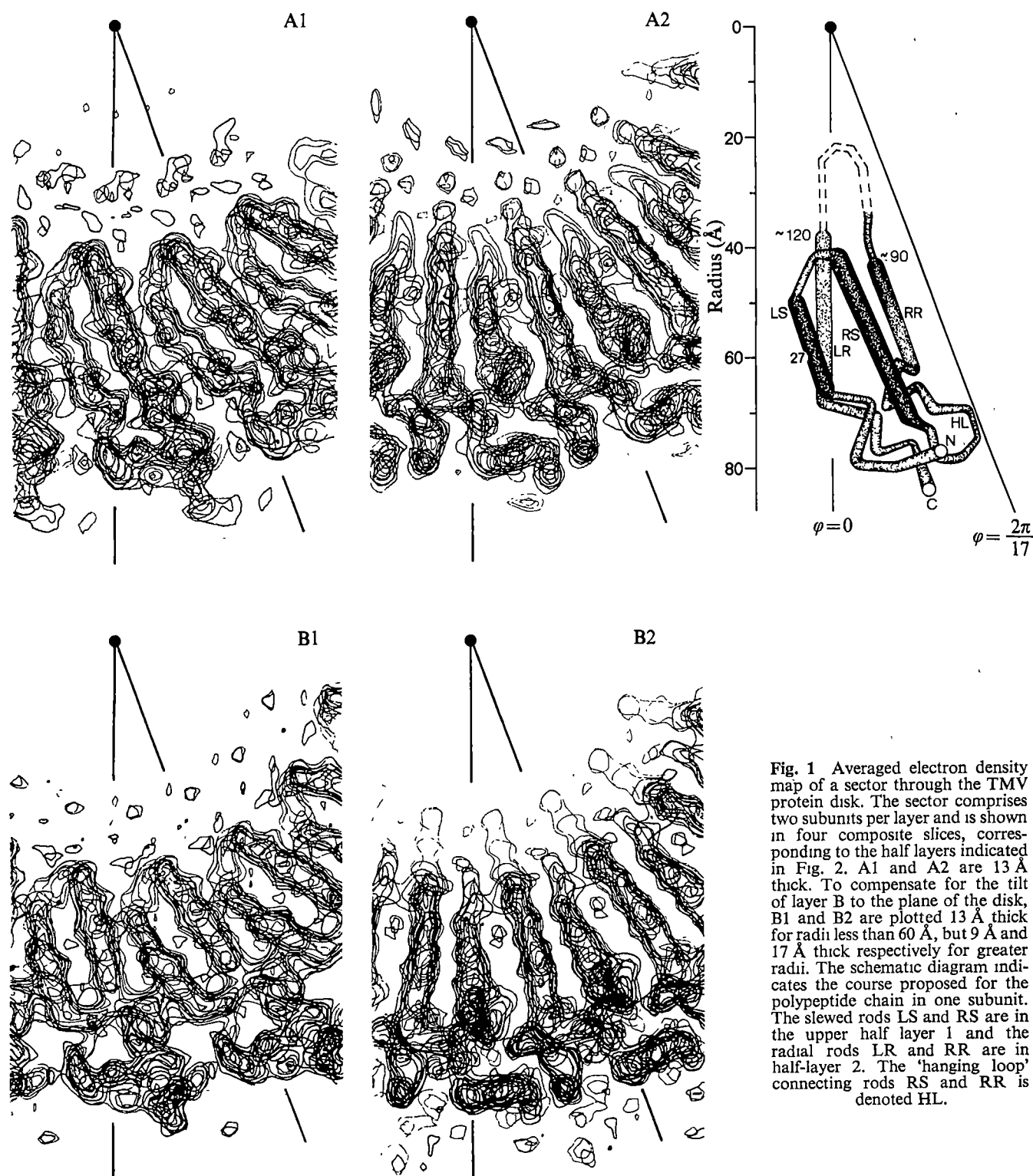


Fig. 1 Averaged electron density map of a sector through the TMV protein disk. The sector comprises two subunits per layer and is shown in four composite slices, corresponding to the half layers indicated in Fig. 2. A1 and A2 are 13 Å thick. To compensate for the tilt of layer B to the plane of the disk, B1 and B2 are plotted 13 Å thick for radii less than 60 Å, but 9 Å and 17 Å thick respectively for greater radii. The schematic diagram indicates the course proposed for the polypeptide chain in one subunit. The slewed rods LS and RS are in the upper half layer 1 and the radial rods LR and RR are in half-layer 2. The 'hanging loop' connecting rods RS and RR is denoted HL.

The structural similarity between the two crystallographically independent layers of subunits in the disk is indicative of the high quality of the phase determination. The map confirms earlier results that the disk is polar and has a pairing interaction between layers at high radius<sup>2,9</sup>, as is clearly seen in side view (Fig. 2).

The precise relationship between the two layers is most easily found by comparing features common to each layer in cylindrical sections of the map (for example Fig. 3). The two are related by an azimuthal shift of about 4°, constant for all radii, whereas the axial separation of common features decreases linearly from 30 Å at low radius to 22.5 Å

at high radius, compared with a constant separation of 23 Å in the virus helix. At the present resolution, therefore, the interaction between layers does not involve any appreciable distortion of the subunits, but rather a bodily tilting by 10° of the subunits in one ring with respect to the other. The large gap between the two layers at a radius of 40 Å evidently allows the initial accommodation of the RNA, since the latter is at this position in the virus<sup>16</sup>.

The rods of density between radii 40 Å and 70 Å, are strongly suggestive of  $\alpha$ -helices. They are approximately equispaced, 10 Å apart, within each layer so that there is little distinction between inter- and intramolecular contacts

in this region. The rods make angles of 10–20° with each other in a left-handed relationship, as is appropriate for right-handed  $\alpha$ -helices. The two slewed rods are connected at 40 Å radius, showing that they belong to the same subunit. At high radius there is a 'hanging loop' connecting the right slewed rod (RS) to the right radial rod (RR) beneath it. The bottom of this loop is in close contact with the left radial rod (LR) of the neighbouring subunit. The simplicity of the arrangement of four dense rods extending from 45 Å to 65 Å contrasts sharply with the highly contorted density towards the outside of the molecule. Interpretation in this region is difficult but is helped by having two independent copies of the subunit, one in each layer. The subunit boundaries in the axial direction are particularly easy to locate because of the clear separation both between disks and within a disk. The chain tracing finally chosen is shown in Fig. 1 together with the electron density map. The subunit delineated is compact, tapering at low radius, broadening and bending azimuthally at high radius.

One disappointing feature of the map is the lack of density at radii below 40 Å; in the helical aggregate of TMV protein<sup>17</sup> 20% of the density lies in this region. The radial rods in our map both peter out at 40 Å radius, suggesting that they are connected at lower radii but that this region is disordered in the disk. The absence of density in the map cannot be accounted for by chain cleavage, since sodium dodecyl sulphate (SDS) gels of old crystals show that virtually all the protein is of the correct size. A second possibility, that the 17-fold symmetry holds only at radii higher than 40 Å so that averaging the map smears out the true structure at lower radii, was discounted on inspection of the unaveraged map which shows no extra density at low radius.

A comparison of our map with that of the virus obtained in Heidelberg by Holmes *et al.*<sup>18</sup> reveals reasonable agreement in the gross features. The connections between the helical regions in our protein subunit, however, do not agree with those derived from their lower resolution map. Both maps show four rods of density at intermediate radii, of which the lower two are more or less radial and the upper two are both slewed to the right. We identify our left and right radial rods with ribbons 3 and 2 respectively of the virus map, and our left- and right-slew rods with ribbons 1\* (of the next subunit) and 4, but the connectivity is quite different. Starting from the N-terminus, our order is LS, RS, RR, LR, corresponding to 1\*, 4, 2, 3 in the Heidelberg notation. The differences in interpretation arise because the clear connection between our slewed rods at 40 Å radius is missing in the virus map and because our map shows no connecting density between the radial rods at 70 Å radius in contrast to the strong loop between ribbons 2 and 3 in the virus map. The radial rods in our map pass continuously into the density at high radius, whereas in the virus map the latter forms an isolated 'island'.

### Folding of the polypeptide chain

The most reliable information for correlating the course of the polypeptide chain (Fig. 1) with the sequence of 158 amino acid residues comes from the location of residues 1, 27, 115 and 139 labelled by heavy atoms in the virus<sup>18,19</sup> and of residue 27 in the disk. There are also indirect data, reviewed by Durham and Butler<sup>20</sup>, mainly concerning the accessibility of residues and regions of conserved sequence. The conservation of positively charged residues in all known strains and mutants at positions 41, 90, 92 and 113 suggests the proximity of these residues in the virus to the sugar-phosphate backbone of the RNA at 40 Å radius.

Arginine 41 can be assigned to the neighbourhood of the bend at 40 Å connecting the LS and RS rods, since cysteine 27 is located by the mercury atom at a radius of

60 Å close to the LS rod (Fig. 1), while the N-terminus is at high radius<sup>19</sup>. The other conserved arginines are therefore probably situated on the radial rods near the RNA radius. This is consistent with putting the stretch of sequence 90–120 at still lower radii, for which there is indirect evidence<sup>21</sup>, and thus assigning this region to the invisible chain connecting the two radial rods. The interaction of RNA with protein would then involve arginine 41 of the subunit below the RNA and arginines 90, 92 and 113 of the subunit above it.

The availability of the C-terminal residue to carboxypeptidase digestion in the intact virus<sup>21</sup> suggests that this is on the outside, at high radius. Residue 139 can be located approximately between the outer continuations of the radial rods by relating the coordinates of the dimercure acetic acid and methylmercury sites in the virus<sup>18</sup> to the methylmercury site in the disk. We choose the left-hand rod for 139, and thus the right-hand rod for 90, as this gives a more plausible connection of the remaining density than does the reverse. The left-hand radial rod is connected to the C-terminus at high radius through density in the lower half of the subunit.

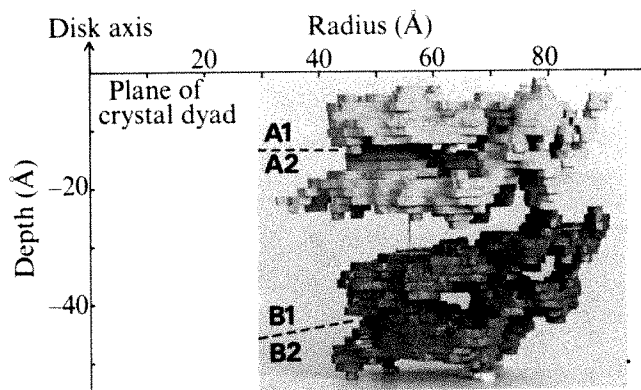


Fig. 2 Side view of a balsa-wood model of two subunits in each layer of the protein disk. The layers interact closely only at high radius, subunits in ring B being tilted away from subunits in ring A at low radius.

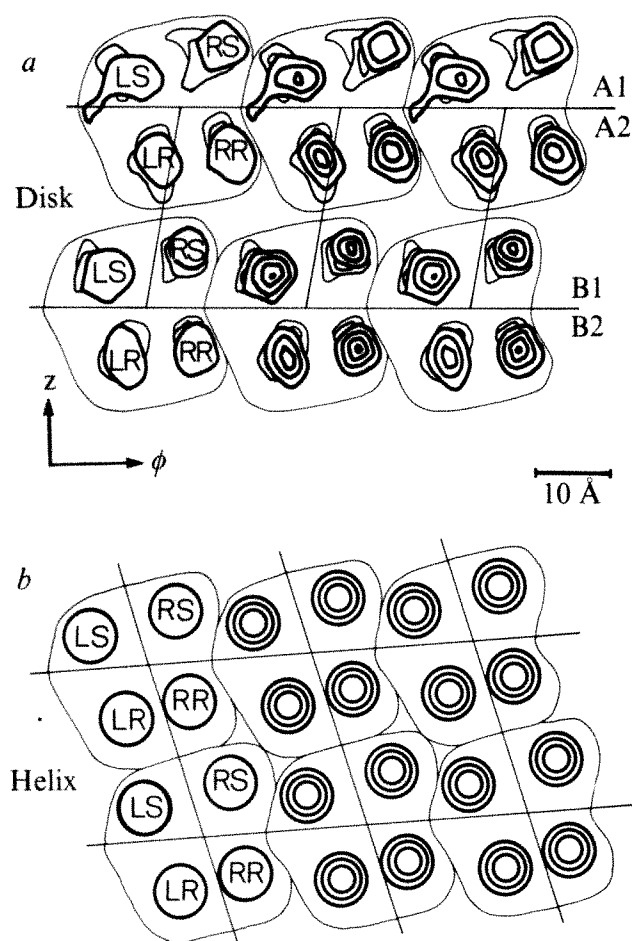
Our interpretation of the map allows the rods to be in the  $\alpha$ -helical configuration over most of their length, and two stretches of sequence rich in hydrophobic residues (74–87 and 119–133) are assigned within the radial rods RR and LR respectively. Parts of the RS and RR rods, however, may be more extended than a regular  $\alpha$ -helix so that the combined length of the two rods and the 'hanging loop' connecting them can be spanned by residues 41–90. The chain tracing proposed here is not the only one conceivable, but accords best with the electron density map. The most uncertain feature concerns a four-way contact at 70 Å radius involving the RS and RR rods and the connecting 'hanging loop'. The direction of the sequence through this loop may be the reverse of that proposed here.

### Subunit packing

The subunits within a layer are tightly packed, the principal contacts being those associated with the  $\alpha$ -helical packing in the region of radii 40–70 Å. There are only two main contacts between layers and these can be seen in Fig. 2 at radii 65 and 80 Å. In the virus, however, lateral and axial contacts between subunits seem to be equally close.

The surface lattices of the disk structure and the virus helix are compared in Fig. 3. The 16-fold helix family in

the virus has a one-third subunit displacement to the left<sup>22</sup> between turns, whereas the two rings of the disk have a relative azimuthal displacement of about one-fifth subunit to the right. Thus the geometry of axial contacts between the two rings of the disk is quite different from that between successive turns of the virus helix. Figure 3a shows a cylindrical section of the electron density map of the



**Fig. 3** Packing of subunits and  $\alpha$ -helical rods in the protein disk and the virus. *a*, Superposed cylindrical sections of the electron density map of the disk at radii 57, 58.5 and 60 Å. The subunit outline is shown. *b*, Schematic diagram of the same subunits arranged in the surface lattice of the virus helix.

disk at a radius of about 58 Å illustrating the packing of the  $\alpha$ -helical rods. The rods are well packed laterally within a layer of the disk, but there is only one close contact between layers. If, however, we generate the packing in the virus in this region by applying the appropriate displacement to one layer such that the surface net of the disk is converted to that of the virus (Fig. 3b), the layers now fit snugly. Further contacts are made giving a continuous two-dimensional array of close-packed rods.

### Implications for virus assembly

The disk has a primary function in both the initiation of the virus structure and its subsequent elongation<sup>5,6</sup> and the structure we have found suggests how this might occur. The disk is not just a simple piece of virus-like structure without the RNA, but it is an intermediate assembly of protein molecules designed for incorporation of the RNA before subsequent transformation to the final helical

arrangement. The contacts between layers of the disk are few, and at the RNA radius the subunits of the disk are about 7 Å further apart axially than they are in the virus. The two layers of the disk thus have the appearance (Fig. 2) of an open pair of 'jaws', awaiting the incorporation of the RNA. We would expect initiation of assembly to involve the reaction of a single disk with the RNA, as has been found to be the case (ref. 6, p. 343).

As the RNA binds to the disk at 40 Å radius, the inner disordered region presumably adopts the virus configuration, the bonds between layers at higher radius being broken as the new bonds characteristic of the virus configuration (compare with Fig. 3) are formed. The disk would presumably dislocate to a helical segment at some point in this process, the RNA beginning its second turn. Another disk could then attach to start the regime of rod elongation. There is no need to postulate distinct mechanisms for initiation and elongation; the flexibility of the chain at low radius would facilitate intercalation of the RNA between the protein subunits, thus resolving the paradox of elongation from a two-layered structure—the disk<sup>22</sup>.

The disorder of the protein in the disk at low radius may be symptomatic of a somewhat nonspecific chain configuration at low radii in the virus, where a unique protein sequence has to accommodate a variety of RNA base triplets in different subunits. This disorder in the disk suggests that the protein does not carry three preformed sites, of the lock-and-key kind, to house the RNA, but rather that the sites are created during the interaction between them. Nevertheless, there must be sufficient stereospecificity in the disk to recognise the special initiation sequence of the RNA, although not all successive bases of this sequence need be involved.

Further details of the interactions between subunits and between protein and RNA, together with confirmation of the folding we propose for the protein subunit must await a 2.8 Å resolution map, the data collection for which is now in progress.

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# letters to nature

## Pulsar atmospheric current loops

THE basic difficulty with the theories of pulsar radiation is that there is as yet no self-consistent model of the large scale structure of the plasma atmosphere of the pulsar<sup>1, 2</sup>. The lack of such a model is perhaps most strikingly exemplified by the fact that there is no consistent explanation of how an aligned rotating magnetised neutron star can maintain a net zero current from its surface—that is, how either a finite current loop in its atmosphere, or an 'infinite' loop to the nebula, is established consistent with the space charge and the electromagnetic fields<sup>3, 4</sup>. This letter will be limited to a discussion of how current loops are established when the rotational and magnetic axes are aligned.

To avoid the extreme conditions which may exist in real pulsars, we will use a gedanken device to tackle the problem. The rotational state of the star will be obtained by beginning with a magnetised star in a vacuum, and adiabatically increasing its angular velocity  $\Omega(t)$ . In this way the various physical complications can be introduced in a systematic manner—ordered according to the value of  $\Omega$ . Our concern is essentially limited to the lowest order influence of  $\Omega$ , which requires a resolution of the current loop problem.

The rotation of a conducting star about its dipole magnetic field axis, induces an electric field which must satisfy the boundary condition

$$E_\theta(r = R, \theta) = -(Q/2R^2) \sin 2\theta \quad (1)$$

where  $Q = (\Omega \cdot \mathbf{B}_0)R^3/c$ , and  $\mathbf{B}_0$  is the polar magnetic field strength. Goldreich and Julian<sup>3</sup> recognised that for real pulsars this yields very large values of  $\mathbf{E} \cdot \mathbf{B}$ , causing large currents from the star, hence invalidating the vacuum assumption. They then made the assumption that  $\mathbf{E} \cdot \mathbf{B} \approx 0$  which, together with equation (1), leads to a corotational atmosphere and their paradoxical result concerning 'proton' currents passing through

negatively charged regions<sup>4</sup>. They also introduced the assumption of a group of open magnetic surfaces, along which current flows to the nebula. These basic assumptions, which have been widely accepted in the literature, are totally different from the model proposed here.

If we take  $\Omega$  to be small (hence small  $Q$ ), the Goldreich and Julian vacuum field would initially draw only electrons from the star, primarily from the polar regions. This would give rise to a stellar charge  $q_s = \epsilon Q$ . To the degree that the electrons move to large distances, thus producing little space charge, the electrostatic potential which satisfies equation (1) is

$$\Phi = \frac{Q}{r} \left[ \epsilon - \frac{1}{6} \left( \frac{R}{r} \right)^2 (3 \cos^2 \theta - 1) \right] \quad (2)$$

and

$$\mathbf{E} \cdot \mathbf{B} = \frac{QB_0}{R} \left[ \epsilon - \left( \frac{R}{r} \right)^2 \cos^2 \theta \right] \left( \frac{R}{r} \right)^5 \cos \theta \quad (3)$$

The effect of the stellar charge is to slow down and eventually stop any net electron escape from the star. This will occur approximately when the polar potential equals the nebular potential at some nebular distance ( $r = R_N$ ). For  $R_N \approx \infty$ ,  $\Phi(R, \theta = 0) = \Phi(\infty, \theta = 0)$  if  $\epsilon = 1/3$ . This net stellar charge,  $q_s = Q/3$ , does not stop electron emission from the polar region, as can be seen from equation (3). The problem therefore remains to determine the flow of these electrons which can no longer escape into the nebula. The details of this plasma flow is not particularly simple, and will be discussed in a later publication. To give a qualitative (and simplistic) picture, it is of some help to introduce regions in which the plasma flow is dominated by either the electric or magnetic field:

$$\begin{aligned} \text{magnetic flow region (MFR): } |\mathbf{E} \times \mathbf{B}| &> E^2 \\ \text{electric flow region (EFR): } |\mathbf{E} \times \mathbf{B}| &> B^2 \end{aligned} \quad (4)$$

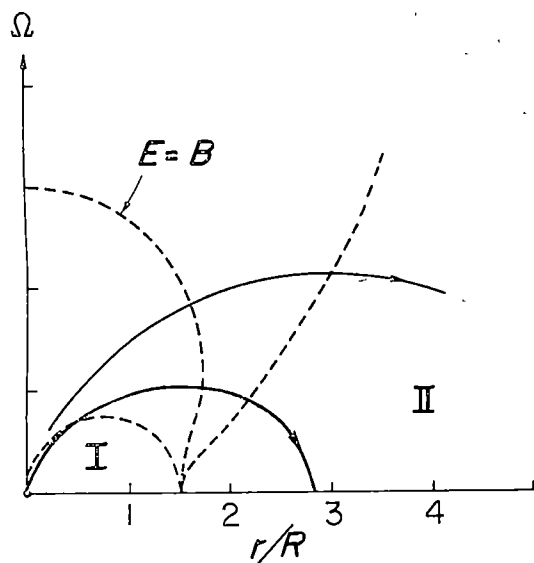
In these regions the average acceleration of the particles is along  $\mathbf{B}$  (in the MFR) or  $\mathbf{E}$  (in the EFR), and in an intermediate direction in the region near  $\mathbf{E} = \mathbf{B}$ . These regions are illustrated in Fig. 1 for the dipole  $\mathbf{B}$  field, and  $\mathbf{E}$  obtained from equation (2), with  $\epsilon = 1/3$ . The (toroidal drift velocity/ $c$ )  $\equiv \beta$ , given in ref. 5 by  $\beta/(1 + \beta^2) = (\mathbf{E} \times \mathbf{B})/(E^2 + B^2)$ , is not corotational and generally  $\beta \neq 1$  any place on a field line. The essential point to note is, however, the flow across magnetic surfaces in the EFR (a point which apparently has not been noted in the force-free approximation).

If electrons start from rest at  $(R, \theta_0)$  and move out along the dipole field lines  $r = R(\sin \theta / \sin \theta_0)^2$  (Fig. 1), where the field line is labelled by its stellar surface angle  $\theta_0$ , they would be energetically able only to reach a point  $(r^*(\theta_0), \theta^*(\theta_0))$  given by

$$\Phi(r^*, \theta^*) \equiv \Phi(R(\sin \theta^* / \sin \theta_0)^2, \theta^*) = \Phi(R, \theta_0) \quad (5)$$

In this case, once arriving at  $\theta^*(\theta_0)$  they would be reflected and return to the star. If, however,  $\theta^*(\theta_0)$  falls in the EFR, the electron will accelerate across magnetic surfaces to surfaces with larger values of  $\theta_0$  (lower electrostatic energy). [Note that this fixation on the EFR is a dynamic simplification, which neglects some 'shorting' across magnetic surfaces which begins when  $E > B$  (Fig. 1). The present concern is conceptual rather than

Fig. 1 Plasma flow regions: acceleration along  $\mathbf{B}$  for the MFR (region I), along  $\mathbf{E}$  for the EFR (region II), or in intermediate directions ( $E \approx B$ ). The star is at the origin. The solid lines follow dipole lines:  $r = R(\sin \theta / \sin \theta_0)^2$ .



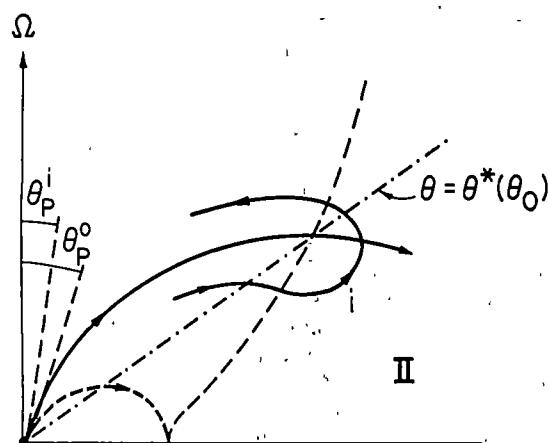


Fig. 2 Schematic of the primary electron current loop which crosses magnetic surfaces in the EFR. Inner and outer polar angles ( $\theta_p^i$ ,  $\theta_p^o$ ) at the stellar surface are defined by the indicated magnetic surfaces.

analytic.] This, then, distinguishes three groups of magnetic surfaces: (a) those such that  $(r^*(\theta_0), \theta^*(\theta_0))$  lies in the EFR; (b) those surfaces which enter the EFR, but for which  $(r^*, \theta^*)$  is not in EFR; (c) those surfaces which do not enter the EFR. The stellar surface angle which separates the group (a-b) and (b-c) define an inner polar angle ( $\theta_p^i$ ) and an outer polar angle ( $\theta_p^o$ ) respectively. These features are illustrated in Fig. 2.

This model predicts that the electrons which flow out along the magnetic surfaces  $\theta_0 < \theta_p^i$  will enter a region (for example the EFR) where they cross magnetic surfaces to surfaces with larger  $\theta_0$ . In the process they will leave the electric-dominated region and then follow their new magnetic surface back to the star—forming the required current loop. The return flow of electrons will be largely restricted to the stellar annulus  $\theta_p^o > \theta_0 > \theta_p^i$ , the larger angle corresponding to the outermost surface to enter the EFR. An examination of the potential energy obtained from equation (2) shows that this flow pattern is consistent with the fields. This flow is schematically illustrated in Fig. 2. It will be noted that this current loop does not extend to the nebula, and does not represent any particle loss from the star (in contrast with large particle losses assumed in many pulsar models).

The present results are only valid to lowest order in  $\Omega$ . The use, however, of an adiabatically increasing  $\Omega(t)$  not only solves the immediate problem, but also gives a number of interesting side-features. The simple current diagram in Fig. 2 in reality consists of a 'plume' or 'fountain' particle flow formation, which cannot be described by simple hydrodynamic equations, and is quite likely unstable. Moreover, equation (2) exhibits a saddle-point potential energy minimum above the poles, which can naturally account for a stationary plasma atmosphere. The gedanken approach shows that this atmosphere, while requiring more stellar electrons, will not change the net star-plus-atmospheric charge, and thus the long range Coulomb force on which the present results rest. These features of a more complete pulsar atmospheric model will be published in the near future.

Finally it should be noted that the details of our results will be significantly changed if the rotational and magnetic axes are not aligned. This is clear from the spatial scale shown in Fig. 1. The analysis of the non-aligned case will be presented in a separate paper.

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## An interstellar H<sub>2</sub> indicator in direction of the Crab Nebula

MOLECULAR hydrogen is very stable at low temperatures, and is therefore likely to be found in large quantities within interstellar dark clouds which are not penetrated by ultraviolet radiation (which destroys molecular bonds).

A case for a high concentration of H<sub>2</sub> in the interstellar medium can be made on the observed distribution of the galactic  $\gamma$ -ray emission<sup>1,2</sup>, but available information is still rather scanty as direct evidence of its presence comes only from regions which are very close to the Earth. For regions further out than 1 kpc, the experimental evidence is only indirect—obtained from CO microwave emission.

We have studied the shape of the low energy spectrum of galactic X-ray sources detectable on Earth. In the energy range 0.1–1 keV the continuous absorption by H<sub>2</sub> is intense. It adds to that produced by other components of the interstellar medium and affects the depth of the absorption edges of elements such as oxygen, neon and others. The oxygen feature at 0.532 keV is very prominent and allows a sensitive probe of the H<sub>2</sub> density between the X-ray source and the observer. We therefore calculated how such features in the low energy X-ray spectrum of the Crab Nebula would be affected by the interstellar abundance of molecular hydrogen.

H<sub>2</sub> between us and the Crab would not affect the propagation of the 21 cm radiation<sup>3</sup>, but we find that it would absorb X-rays. This accounts for a factor of 2 in the difference between X- and radio-estimations of the column density of the interstellar medium in this direction. Indeed, the higher X-ray column density cannot be explained by interstellar grains or by any anomalous abundance of the usual components of interstellar matter, except on very specific models which lack any experimental support<sup>4-6</sup>.

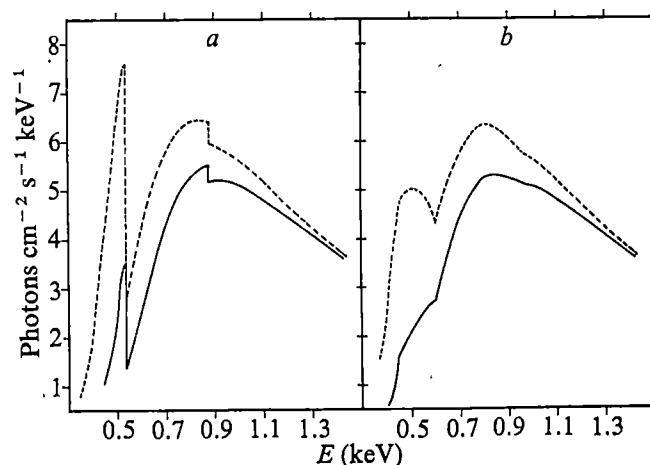
We made the following assumptions in our calculation: first the X-ray energy spectrum of the Crab Nebula in absence of absorption follows a power law

$$N(E)dE = KE^{-\gamma}dE$$

of constant spectral index  $\gamma$ .

Extrapolation to low energy of the spectrum measured above a few keV, where absorption is negligible, gives  $\gamma = 2.0$

Fig. 1 The X-ray energy spectrum of the Crab Nebula modified by interstellar absorption as seen with infinite resolution,  $\Delta E = 0$ , (a), and finite resolution,  $\Delta E = 150$  eV, (b), for different values of H<sub>2</sub> abundance. — — —,  $R = 0$ ; ———,  $R = 0.5$ .



**Table 1** Depth  $D$  of the oxygen absorption edge as a function of the  $H_2$  abundance  $R$ 

$R$	$D(R)/D(0)$
0	1
$5 \times 10^{-3}$	0.996
$10^{-2}$	0.980
$3 \times 10^{-2}$	0.948
$10^{-1}$	0.856
$3 \times 10^{-1}$	0.628
$5 \times 10^{-1}$	0.460
$7 \times 10^{-1}$	0.337

(refs 4, 5, 7). Second, the column density of atomic hydrogen is  $2.0 \times 10^{21}$  atoms  $\text{cm}^{-2}$  compatible with radio measurements at 21  $\text{cm}^8$ . Third, except for molecular hydrogen, the composition of the interstellar medium is standard. The absorption cross sections and abundances computed by Brown and Gould<sup>9</sup> have been adopted, and fourth, in the energy range of interest the absorption cross section of  $H_2$  has the form

$$\sigma_{H_2}(E) = 1.84 \times 10^{-22} E^{-3.3} \text{ cm}^2$$

Results of the calculations are presented in Fig. 1 and Table 1. Figure 1a shows the expected Crab Nebula low energy X-ray spectrum assuming  $R = 0.0$  or  $R = 0.5$  (where  $R = 2N_{H_2}/N_H$ ,  $N_{H_2}$  and  $N_H$  being respectively the densities of molecular and atomic hydrogen) and infinite resolution, ( $\Delta E = 0$ ). When  $H_2$  is present in the interstellar medium the depth of the absorption edges decreases. Table 1 gives, for various  $H_2$  abundances, the depth of the oxygen feature divided by the depth of the feature when  $R = 0$ . Figure 1b gives the same spectra as Fig. 1a calculated under the hypothesis that the best energy resolution now available at 0.5 keV is used,  $\Delta E = 150$  eV. The neon edge at 0.874 keV practically disappears. The oxygen absorption edge, indicated by a bump in the smooth trend of the spectrum, is now the only feature which stays

**Table 2** Height  $H$  of the oxygen bump in units of  $h\sigma$ , (see text), as a function of the  $H_2$  abundance  $R$ 

$R$	$H/h\sigma \text{ (cm}^2 \text{ s)}^{1/2}$
0	1.47
$10^{-2}$	1.46
$3 \times 10^{-2}$	1.44
$10^{-1}$	1.36
$3 \times 10^{-1}$	1.17
$5 \times 10^{-1}$	1
$6 \times 10^{-1}$	0.92

visible. To detect it in an experiment carried out with a detector of area  $A$ , efficiency  $\eta$  and energy resolution  $\Delta E$ , the Crab must be observed for such a long time  $t$  that the bump stands up by at least  $3\sigma$  above the continuum.

Table 2 gives the height of the oxygen bump in units of  $h\sigma$  where

$$h = (g(\Delta E)\eta At)^{1/2}$$

and  $g(\Delta E)$  takes into account the energy resolution of the detector.  $g(\Delta E) \leq 1$  and tends to 1 when  $\Delta E \rightarrow 0$ ; a rough estimate based on comparison between Fig. 1a and b gives  $g(150 \text{ eV}) \simeq 0.3$ . In the present experimental situation ( $A \geq 100 \text{ cm}^2$ ,  $t > 60 \text{ s}$ ,  $\eta \simeq 0.2$ ) values of  $h > 10 \text{ (cm}^2 \text{ s)}^{1/2}$  are common and  $h \simeq 1,000$  possible (on satellite experiments). The oxygen feature is therefore a sensitive indicator of the molecular hydrogen abundance in direction of the Crab Nebula.

At present, data on the soft X-ray flux from the direction of the Crab Nebula are available only at energies near and above 1 keV, where the absorption effects are completely within the present experimental uncertainty of the measured flux. We cannot therefore carry out comparisons. In the near future,

however, observations particularly aimed at observing the oxygen feature, (Griffiths, personal communication), will extend the available information towards lower energies and allow significant comparison between theory and experiment.

Some comments are necessary about the  $H_2$  absorption cross section. Between 0.1 and 1 keV there are estimates of  $\sigma_{H_2}$  which differ by a factor as high as 1.5 (ref. 10) from the one we used and give rise to an oxygen absorption edge higher by up to a factor  $\simeq 2$  at  $R = 0.5$ . In the absence of firm theoretical and experimental indications, we decided to use the most widely adopted cross section value in order to obtain results easily comparable with other calculations.

In spite of such an uncertainty, comparisons between calculated and measured spectra of the Crab Nebula X-ray flux are still worthwhile.

Application of this method to other X-ray sources, for which observations at sufficiently low energy already exist (see for instance ref. 11), can be made provided that first, the source distance or the hydrogen column density in the source direction is known and, second, reasonable assumptions on the source X-ray spectrum in absence of absorption are possible.

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## Transient short time periodicities in the optical emission from Cyg X-1

TEMPORAL structure down to the millisecond time scale has been observed in the X-ray emission of Cyg X-1 (ref. 1). This has been interpreted as evidence of instabilities in the accretion disk surrounding a black hole<sup>2-4</sup>, lasting for approximately the orbital period of the infalling matter. We report here observations of modulated optical emission from this source with a period of  $\sim 83$  ms. The modulated emission was detected in two sets of observations as transient events lasting  $\sim 10$  min. This is the first evidence of strong emission at optical wavelengths from the X-ray source in the Cyg X-1-HDE226868 system.

The short period variations in optical emission were investigated with a photometric arrangement on the 91-cm telescope at the Catania Astrophysical Observatory. Single photon pulses and 1 kHz reference signal were recorded on magnetic tape, and the counting rate for 1-ms intervals subsequently transferred to digital tape. Computer analysis included correction for variable atmospheric absorption, Fourier analysis with the Cooley-Tukey (FFT) algorithm and count rate folding. The time base accuracy is 1 part in  $10^8$ , achieved by daily checking against the BIH international time scale using a 5-MHz radio link.

Systematic observations of both comparison stars and Cyg X-1 (when quiet) confirms that scintillation noise cannot

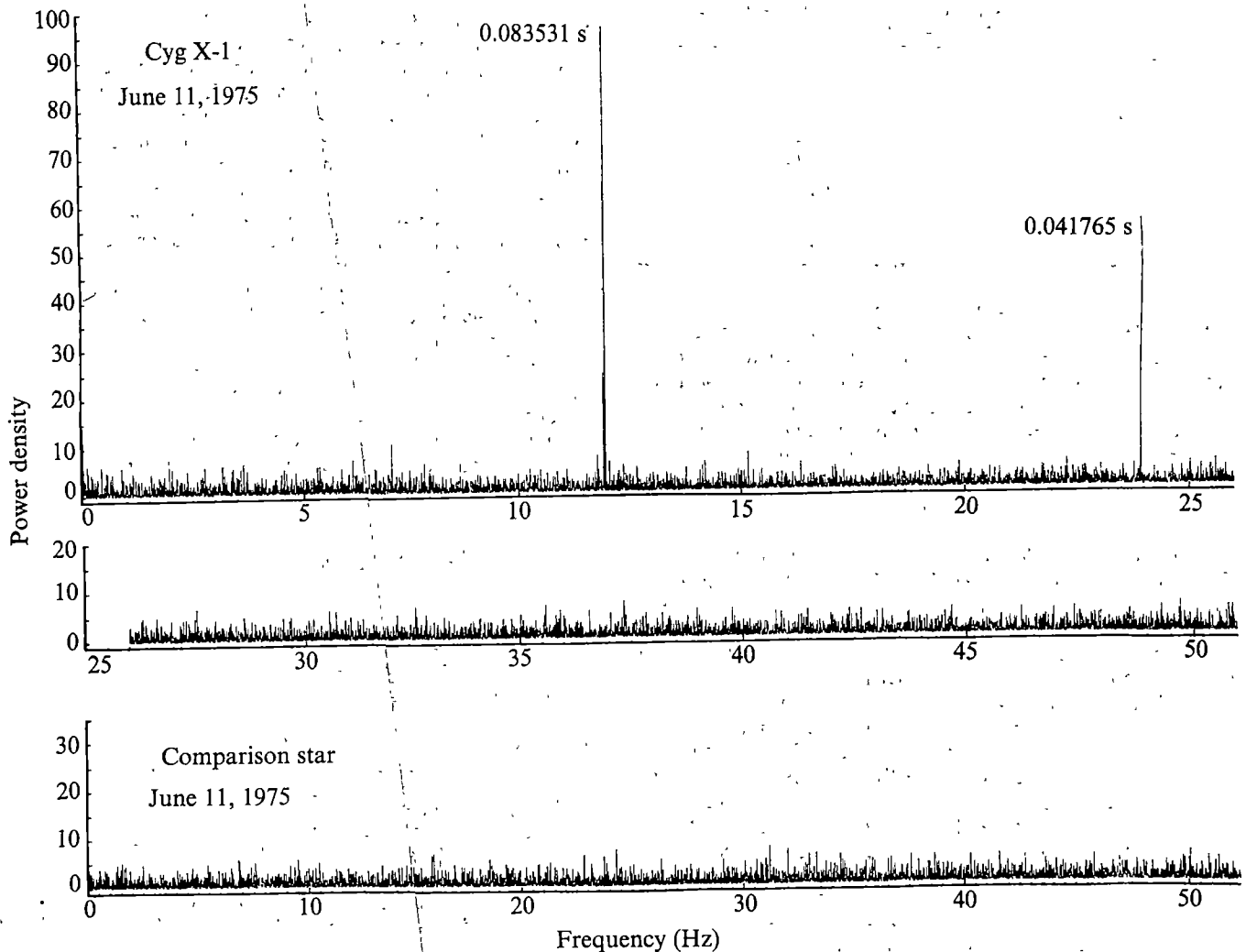


Fig. 1 Power density spectrum of the event of June 11.06111 and of the comparison star.

simulate a statistically significant modulation above a few Hz. The statistical distribution of the fluctuations in the Fourier spectrum is quite consistent with that expected from counting statistics, and we therefore deduce a sensitivity for our survey of 0.1% pulsed fraction with 99% statistical confidence in the frequency range 5–500 Hz.

Routine observations of Cyg X-1 were carried out in November 1973 and September 1974 with negative results. Observations were repeated in June 1975 after the observation of a new flare by the X-ray equipment on the *ANS* satellite<sup>5</sup>. Analysis of the data has revealed that a strong pulsed component appeared at 01.28 UT, June 11. The pulsation with a period of  $83.531 \pm 0.008$  ms lasted for  $\sim 10$  min. After this event no significant power was detected in the Fourier spectrum of Cyg X-1, nor in the check performed on the comparison star soon afterwards (Fig. 1).

The light curve of the pulsation, obtained by folding the counting rate module 83.531 ms, shows a very regular structure. The periodicity detected by Fourier analysis is caused by falls of intensity with a duty cycle near 25% (Fig. 2) naively suggesting an eclipsing phenomenon.

Figure 3 shows the time evolution of this event in steps of 30.72 s from the beginning of the tape to the merging of the pulsation in the background fluctuations. In this plot we have a clear picture of the settling of the pulsations. In fact, we observe that the modulated component is superimposed on the steady-state luminosity of the source. This brightening can be

also observed in a plot of the counting rate integrated for 1 s. On this plot the modulation growth has a rise time of  $\sim 3$  s. It should be noted that the modulation period was not strictly

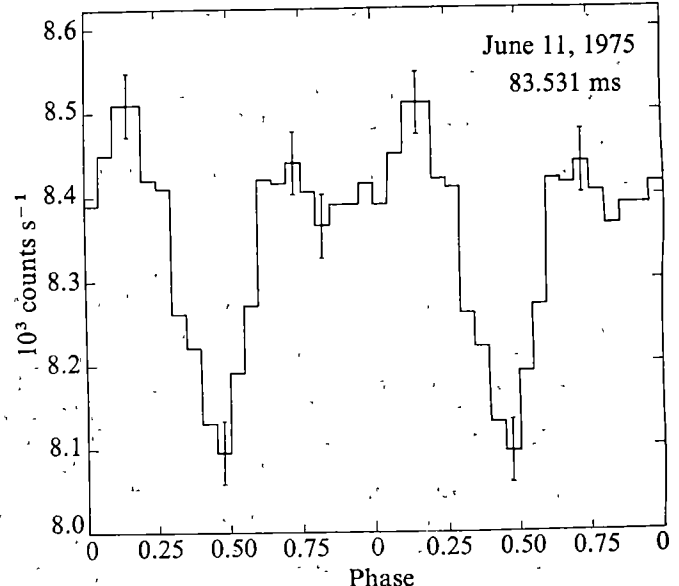


Fig. 2 Light curve of the modulated emission, obtained by folding  $\sim 100$  s of data.

Table 1 Data from flare events on Cyg X-1

Time	Period $P$ (ms)	$\Delta P/P$	Filter	Duration (min)	$\Delta L/L$
June 11.06111 UT	$83.531 \pm 0.008$	$10^{-4}$	Grey ( $\times 10$ )	10	3.5 per cent
July 18.00410 UT	$83.714 \pm 0.008$	$2 \times 10^{-4}$	R	> 10	2.5 per cent
July 18.03674 UT*	$83.592 \pm 0.008$	$\geq 2 \times 10^{-4}$	B	> 6	3.7 per cent
July 18.04854 UT*	$83.592 \pm 0.008$	$\approx 2 \times 10^{-4}$	Grey ( $\times 10$ )	> 8.5	4.2 per cent

\*Probably related to the same event.

constant during the event, as shown by a significant variation in the phase of the minima. We estimate that during the event the period fluctuated by  $\sim 1$  part in  $10^4$ .

A similar activity in the source was observed one month later. On July 18 several events occurred in the space of  $\approx 1$  h. The data are given in Table 1. The light curves of the events in

because of the large luminosity of the spots which could come only from a non-thermal process.

Alternatively, if the phenomenon is associated with a rotating body, one cannot explain the variations of the period observed without assuming a triple star. In this case the period variability implies an orbital velocity  $> 300 \text{ km s}^{-1}$  for the rotating body.

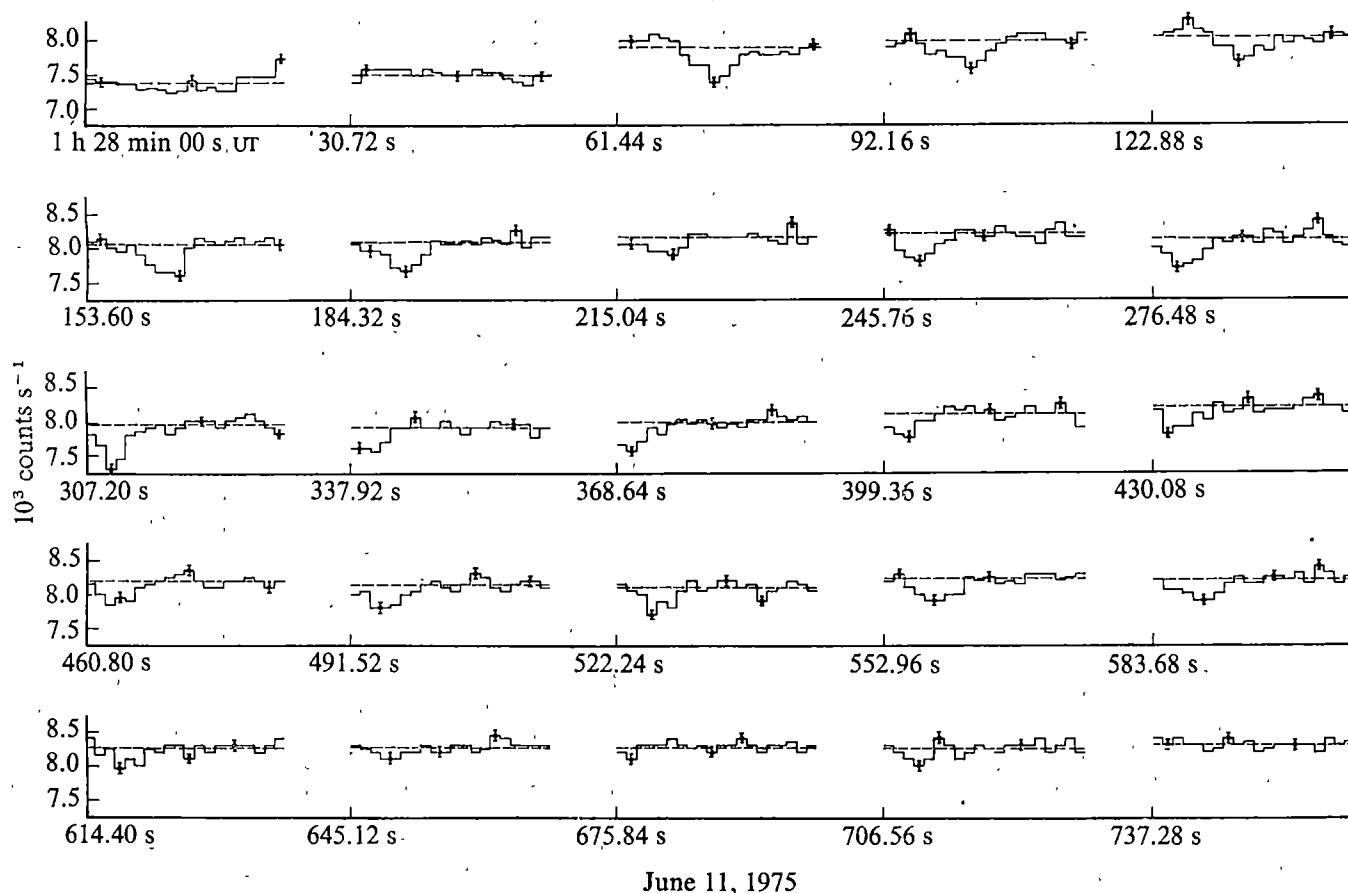


Fig. 3 Evolution of the June event. Each plot corresponds to the folding of  $\sim 30$  s of data.

July are quite similar to those of June except that the duty cycle of the drop in intensity is here closer to 50%. Some ambiguity can arise in the time separation of the events. We assumed that the period given by Fourier analysis (the mean period) identifies each individual event. We stress that differences in period are quite noticeable, although small ( $\sim 1$  part in  $10^9$ ). The luminosity of the modulated emission can be estimated assuming that the bulk of the optical luminosity comes from the supergiant blue star HDE226868, and that this has the absolute visual magnitude of  $-6.5$ , corresponding to its spectral type. This gives a surprisingly high luminosity for modulated emission  $\sim 10^{35}$ – $10^{36} \text{ erg s}^{-1}$ .

Very qualitatively, such a periodicity in the optical emission could fit the popular accretion disk models for Cyg X-1. We may assume that this optical emission is generated by instabilities in 'spots' in the disk. Our data would then indicate that such instabilities arise in a rather limited region at a distance of a few hundred kilometres from the black hole. Difficulties arise

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## Entropy production by black holes

HAWKING has shown<sup>1</sup> that black holes, treated quantum mechanically, emit black body radiation of temperature  $T = 10^{-7} (M_{\odot}/M) \text{ K}$ , and hence evaporate—if isolated—on a time scale<sup>2</sup> of  $\sim 10^{66} (M/M_{\odot})^3 \text{ yr}$ . Energy arguments<sup>1,3,4</sup> suggest that a black hole can be assigned the entropy

$$S_{\text{bh}} = 4\pi GM^2 k / \hbar c \sim 10^{77} k \sim 2 \times 10^{18} S_{\text{H}}(M/M_{\odot})$$

where  $S_{\text{H}} \sim 40(M/M_{\text{p}})k$  is the entropy of a hydrogen cloud from which the black hole is assumed to have formed,  $M_{\text{p}}$  is the mass of the proton, and  $k$  is Boltzmann's constant. This implies that when a hydrogen cloud of mass  $1M_{\odot}$  collapses into a black hole, the entropy of the (uncollapsed) galaxy [ $\sim 10^{12} S_{\text{H}}(M_{\odot})$  and more] would increase by a factor  $\sim 10^6$ . In other words: something hardly observable, namely the collapse of one star, would increase the entropy of our cosmic neighbourhood by a large factor. We endeavour to resolve this apparent puzzle.

To begin with, it must be remembered that in 'normal' conditions, matter has an entropy particle  $s \sim 1$ , ( $s = SN^{-1}k^{-1}$ ) where  $N$  = particle number;  $s$  varies between  $\lesssim 1$  (for example for electrons at temperatures  $T$  below the Fermi temperature  $T_{\text{F}}$ ) and  $\lesssim 90$  (for dispersed hydrogen of critical cosmological density  $a \sim 10^8 \text{ K}$ );  $s \sim 4$  for an extreme relativistic ideal gas. When a star forms by contraction of a cloud,  $s$  ( $\sim -\ln(n\lambda^3) + 5/2$ ) decreases from  $s_{\text{cloud}} \sim 40$  to  $s_{\text{star}} \sim 10$  (where  $n$  = particle number density,  $\lambda = h/(2\pi mkT)^{1/2}$  is the thermal de Broglie wavelength), but the second law of thermodynamics is not violated because the initial entropy has been converted into thermal radiation. If the star continues to shrink, its entropy

decreases still further, and again the excess entropy goes into thermal radiation. Following the matter through, we find a black hole of  $1M_{\odot}$  to have a rather small relative entropy at formation smaller than that of a neutron star. On the other hand, an isolated black hole evaporates within  $t_{\text{evap}} = 10^{66} (M/M_{\odot})^3 \text{ yr}$  and thereby emits black body radiation of temperature  $T$ . For complete evaporation, the vacuum must be large enough to take this black-hole energy. In filling this volume, the radiation randomises and acquires precisely the entropy given above; which is  $10^{18} (M/M_{\odot})^2$  times larger than that at formation.

This suggests the following resolution of our puzzle: a black hole forms on the collapse time scale  $t_{\text{coll}} = GM/c^3 = 10^{-5} (M/M_{\odot}) \text{ s}$ , with an entropy at birth smaller than that of its building blocks. Thereafter, if left alone it irreversibly evaporates on the (huge) time scale  $t_{\text{evap}}$ , with a (small) entropy production rate  $S \sim S_{\text{bh}}/t_{\text{evap}}$ . This implies that the entropy of the Galaxy is hardly affected by black hole formation. The so-called entropy of a black hole is the entropy it would produce during its irreversible evaporation.

Our suggested resolution can be made more explicit by noting that the entropy of an arbitrary thermodynamic system is defined as a hypersurface integral over the entropy four-current density  $s^a$ ,

$$k^{-1} S[\Sigma] = \int_{\Sigma} s^a dx_a^*$$

with  $s^a$  a future time-like vector inside the substratum (third law),  $s^a = 0$  in vacuum, and  $s^a \geq 0$  throughout (second law). In flat space-time,  $\Sigma$  is to be the hyperplane normal to the observer. In an asymptotically flat space-time,  $\Sigma$  will be chosen tangential to a distant observer's normal hyperplane; but in general there is a continuous arbitrariness in the choice of  $\Sigma$  in non-static domains. Such an arbitrariness implies that there will be a minimum and a maximum entropy for each space-time point  $x$ , namely the above functional  $S$  for the 'earliest' and the 'latest'  $\Sigma$  through  $x$ .  $S_{\text{min}}(x)$  and  $S_{\text{max}}(x)$  are equal for  $s^a = 0$  in the enclosed domain, that is, if all thermodynamic processes taking place are reversible, and almost equal for slow processes. An extreme situation occurs when a black hole forms, because then  $\Sigma$  should avoid some ill-understood space-time domain inside the hole: at certain (distant) observer times,  $\Sigma$  must cut through the black hole interior which cannot be observed in the classical (non-evaporation) limit. But the Cauchy development makes unique predictions (in principle) which can be used to calculate  $S[\Sigma]$ . And a glance at Fig. 1 shows how a distant observer notices the evaporation of an isolated black hole. In this two-dimensional conformal space-time diagram, light rays propagate at  $45^\circ$ ; angular degrees of freedom are suppressed; the collapsing star is represented by some of its (time-like) worldlines including the worldline of its centre (with a classically singular portion marked '?'). Flashes of radiation are emitted during the collapse and at the end of evaporation (when  $T(M)$  diverges, and a singularity forms). The entropies in the consecutive space sections  $\Sigma_1, \Sigma_2, \Sigma_3$  are of order  $S_{\text{H}}, \alpha S_{\text{bh}}, S_{\text{bh}}$  respectively, where  $\alpha$  is smaller than, but of the order of, unity.

These statements are at variance with those of Hawking<sup>5</sup> or earlier authors<sup>6</sup> who assign a time-independent value  $S_{\text{bh}}$  to the entropy contained inside the event horizon of a black hole, and which I consider untenable for the reasons given above. In particular,  $S_{\text{bh}}$  is  $10^{18} (M/M_{\odot})^2$  times larger than the entropy determined by local observers inside the black hole! And infalling observers cannot be discarded as 'unphysical': if our Galaxy contracted within its Schwarzschild volume, we would have another month to live under almost present-day conditions—not at almost infinite entropy density—for which period some of us may like to have predictions.

If we accept this, black holes of the same mass, spin, and charge can have different entropies, depending on their ways of

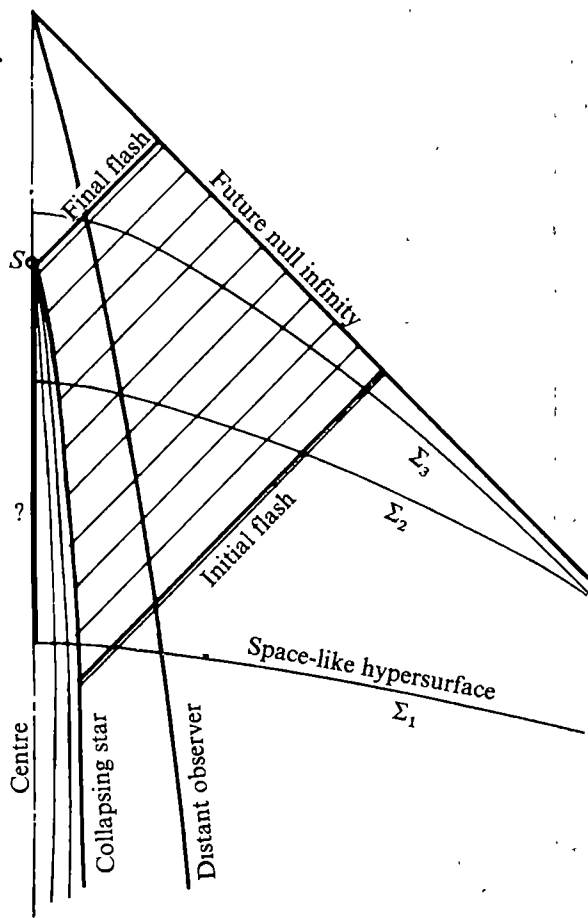


Fig. 1 Conformally finite radial space-time section through an isolated collapsing star (Penrose diagram).

formation. The highest entropy would be obtained for a black hole formed from the coldest possible radiation—radiation at its evaporation temperature. In this case, and only in this case, would evaporation be an isentropic process,  $S_{bh}$  being (already) the entropy at formation. Such black holes can exist in thermal equilibrium with their surroundings, but they cannot form<sup>5</sup> spontaneously from radiation unless their mass is below  $10^{-5}g$ , (corresponding to temperatures above  $10^{32}K$ ); they have to be synthesised from lower entropy ones.

What do we know about the entropy in the Universe? In our cosmic neighbourhood, the comoving entropy is nearly constant and given by that of the 3 K background radiation. Present-day matter contributes<sup>7</sup>, at most, a fraction  $3 \times 10^{-7}$ , (whose slow increase makes life possible). The comoving entropy would be maximal if all matter were converted into heat of uniform temperature; processing through black holes could in principle achieve this as local conservation laws (such as baryon number conservation) are thereby transcended. If this were achieved at time  $t$  ( $\geq t_0$ , the present time), cosmic entropy would be raised by a factor  $\lesssim 10^8(t/t_0)^{1/2}$ . In particular, present-day cosmic entropy is  $\geq 0.1\%$  maximal.

This consideration arose from a conversation with H. Buchdahl, and was promoted by discussions with A. King and J. P. Lasota.

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## Possible implications of the Rubin-Ford effect

AN anomaly in the distribution of radial velocities was reported for Sc 1 galaxies by Rubin *et al.*<sup>1</sup> They found different mean velocities in two nearby hemispherical regions (I and II, see Fig. 1), although the mean apparent magnitude was found to be the same for both sets.

One interpretation they advanced was that the Hubble constant differs in the ratio  $H_{II}/H_I \approx 1.25$  for the two regions, contradicting the cosmologists' canon.

In the RFR<sup>1</sup> paper, an arbitrary division of the Universe into two was made, which has no evident physical significance. A non-cosmological redshift has been suspected for the centres of rich clusters of galaxies, like Coma<sup>2,3</sup>, and the effect noted by Rubin *et al.* may be correlated with this.

To test this hypothesis we divided galaxies into two categories: first, those whose light does not encounter any important cluster of galaxies on its way to the observer (galaxies in region A), and second those situated behind or inside a cluster (region B).

Zwicky's Catalogue of Galaxies and Clusters of Galaxies (CGCG)<sup>4</sup> supplies us with a suitable set of clusters of galaxies. We have limited our study to clusters classified 'near' ( $V \leq 15,000 \text{ km s}^{-1}$ ) whose population was  $\geq 500$  (for 'compact' and 'medium compact' clusters) or  $\geq 1,000$  (for 'open' clusters), so that their density of matter or of radiation is sufficiently high. We added the Virgo cluster which was not included in the CGCG because it is too near. Lastly, to include the possible influence of the gravitational and radiation fields of the clusters, we have included in region B an area around each cluster whose radius is double that of the cluster.

Distances of clusters are given by Noonan's list of published radial velocities<sup>5</sup>. For some clusters not included in that list, estimations were made with three different methods: first we estimated the luminosity classes of Sc and Sb galaxies in clusters<sup>6,7</sup>; this immediately gave us a distance modulus. Second we checked our results for Abell clusters using Abell's method<sup>8</sup>. Third, the Uppsala catalogue<sup>9</sup> supplied us with some other radial velocities.

Since the list of galaxies of Rubin *et al.*<sup>1</sup> is unfortunately not yet published, we used the following: 41 faint radio-sources identified with galaxies<sup>10</sup>. We used all the galaxies in the list within the intervals  $13 \leq m_{\text{corr}} \leq 15.5$ , and  $4,800 \leq V \leq 20,000 \text{ km s}^{-1}$ , and 77 compact galaxies with absorption spectra from Zwicky's Catalogue of Selected Compact Galaxies (CSCG)<sup>11</sup>. The Rubin-Ford effect has already been confirmed for this type<sup>12</sup>. We used the same radial velocity interval as for radio galaxies and revised the list with more simple criteria—we chose all compact galaxies within this velocity interval whose spectra show absorption lines but no emission lines other than [O II]  $\lambda 3,727 \text{ Å}$ , and which lie in the magnitude interval  $13 \leq m_{\text{corr}} \leq 18$ .

In spite of a few criticisms<sup>13,14</sup>, the homogeneity of Zwicky's magnitudes within these limits, seems confirmed by the recent work of Paturel<sup>15</sup>, if a correction is made for declination dependence by the term  $\Delta m_{\text{dec}} = 0.0025 \delta (d^\circ)$ , and if the sample is limited to galaxies of given morphology. These factors do not interfere with our statistical calculations.

**Table 1** Statistical results for the compact galaxies and the radio galaxies

Region		A	B
Radio galaxies	<i>n</i>	21	20
	$m_{\text{corr}}$	$14.62 \pm 0.139$	$14.36 \pm 0.168$
	$V_{\text{km s}^{-1}}$	$9,454 \pm 890$	$11,866 \pm 985$
	HM	$1.01 \pm 0.028$	$1.17 \pm 0.022$
Compact galaxies	<i>n</i>	53	24
	$m_{\text{corr}}$	$15.88 \pm 0.136$	$15.45 \pm 0.194$
	$V_{\text{km s}^{-1}}$	$11,147 \pm 582$	$11,376 \pm 809$
	HM	$0.84 \pm 0.026$	$0.94 \pm 0.033$

*n* is the number of galaxies,  $\langle m_{\text{corr}} \rangle$  is the mean apparent corrected magnitude (see text),  $\langle V \rangle$  is the mean radial velocity relative to the sun and,  $\langle \text{HM} \rangle$  is the mean value of the Hubble modulus.

All apparent magnitudes are taken from CGCG or CSCG. Corrections are also made for galactic absorption by the formula:

$$m_{\text{corr}} = m - A_{\text{pg}} = m - 0.25 \text{ cosec} |b|$$

The two samples of galaxies, with the outlines of region B are plotted in Fig. 1.

To study eventual abnormal redshifts, the best suitable parameter is the Hubble modulus (HM), defined:

$$\text{HM} = \log V - 0.2 m_{\text{corr}} = \log H - 0.2 M - 5$$

where  $H$  is the Hubble constant in  $\text{km s}^{-1} \text{ Mpc}^{-1}$ , and  $M$ ,  $m_{\text{corr}}$ ,  $V$  represent respectively the absolute magnitude, the corrected apparent magnitude and the radial velocity in  $\text{km s}^{-1}$  of a galaxy. With this parameter we can test either a variation of  $H$  (if the mean absolute magnitude has a sufficiently small dispersion) or an eventual deviation of the mean absolute magnitude, provided the distance-symbolic radial velocity ratio is regular enough. The results are given in Table 1, with standard errors.

We would like to make the following comments: first, we obtain the differences  $\langle m_{\text{corr}} \rangle_B - \langle m_{\text{corr}} \rangle_A = -0.26 \pm 0.218$  and  $-0.43 \pm 0.237$  respectively for radio and compact galaxies. These results do not depend on the value chosen for the absorption coefficient, since similar differences are obtained with the non-corrected magnitudes:  $-0.31 \pm 0.212$  and

$-0.33 \pm 0.246$ . Second, the differences of Hubble modulus are contrarily positive and highly significant:

$$\langle \text{HM} \rangle_B - \langle \text{HM} \rangle_A = 0.16 \pm 0.036 \text{ (radio galaxies)}$$

$$\langle \text{HM} \rangle_B - \langle \text{HM} \rangle_A = 0.10 \pm 0.042 \text{ (compact galaxies)}$$

Student's variable is,  $t = 4.4$  and  $t = 2.4$  respectively, which are significant with a quasi-certitude of more than 99.9 per cent and 98 per cent. These results can be compared with the values obtained for the same samples in the case of the subdivision of Rubin *et al.*

$$\langle \text{HM} \rangle_{II} - \langle \text{HM} \rangle_I = 0.02 \pm 0.045 \text{ (radio galaxies)}$$

$$\langle \text{HM} \rangle_{II} - \langle \text{HM} \rangle_I = 0.08 \pm 0.043 \text{ (compact galaxies)}$$

These results suggest the following. First, Sandage and Tamman<sup>14</sup> have suggested that the effect noted by Rubin *et al.* could be explained by a deviation from the normal distribution of absolute luminosity (that is, HM values) 'if the velocity field itself is inhomogeneous (clumped) and if the magnitude limits of the sample are very narrow'. This explanation does not seem available in our case. Indeed  $m_{\text{corr}}$  intervals of our samples are rather large and their redshift distribution is not clumped.

Second, for our samples, the differences of  $\langle \text{HM} \rangle$  in our test are clearly accentuated and concentrated in comparison with those obtained with RFR division. As a consequence the RFR effect should be associated with the difference of distribution of clusters between the two regions of Rubin *et al.*

Third, since the distributions of our galaxies, in both region A and B, are rather uniform on the map of the sky (see Fig. 1), any explanation of the observed effect by a motion of the observer seems to be excluded. This excludes also the explanation by abnormal galactic absorption proposed, for example, by Hartwick<sup>16</sup>. Besides, absorption in the clusters cannot explain the effect, but on the contrary, would increase the difference.

Fourth, it has been suggested that clustering itself could give rise to systematic differences of  $\langle \text{HM} \rangle$ . Indeed absolute magnitude selection effects were reported by van den Bergh<sup>7</sup>. But the systematic difference he found between field galaxies and

cluster galaxies in the Virgo cluster (0.35 absolute magnitude), in the opposite direction to the ones we obtained, favour the underestimation of HM values in the clusters. Furthermore, to test the possible influence of clustering on our samples, we divided them into three regions: C (galaxies belonging to a cluster), A\* and B\* containing the galaxies which remain in the original A and B regions. We found (including standard deviations): compact galaxies:  $\langle \text{HM} \rangle_{B^*} = 0.96 \pm 0.046$  ( $n = 15$ );  $\langle \text{HM} \rangle_C = 0.86 \pm 0.040$  ( $n = 12$ );  $\langle \text{HM} \rangle_{A^*} = 0.85 \pm 0.027$  ( $n = 50$ ); and for radio galaxies:  $\langle \text{HM} \rangle_{B^*} = 1.19 \pm 0.024$  ( $n = 12$ );  $\langle \text{HM} \rangle_C = 1.11 \pm 0.033$  ( $n = 12$ );  $\langle \text{HM} \rangle_{A^*} = 1.01 \pm 0.034$  ( $n = 17$ ). It is seen that  $\langle \text{HM} \rangle_{B^*} > \langle \text{HM} \rangle_C \geq \langle \text{HM} \rangle_{A^*}$  which suggests that the observed effect is not due to clustering.

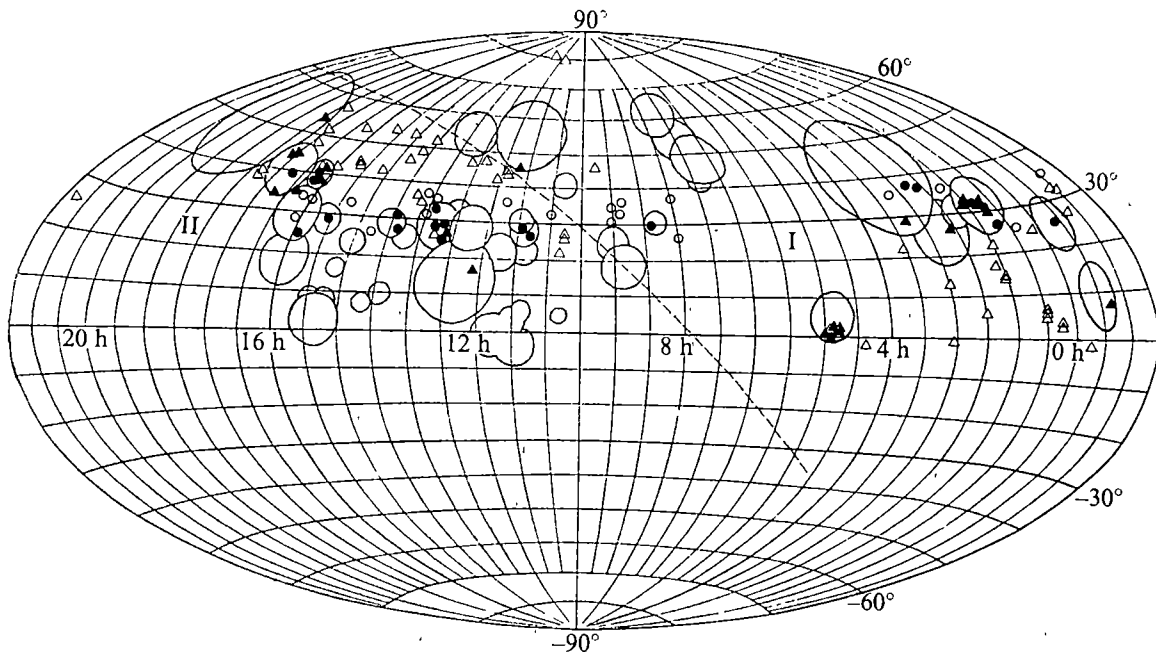
Finally, there remains, of course, the possibility that this phenomenon may be interpreted as Malmquist bias: for a magnitude limited sample, distant galaxies will be overluminous as compared to the mean. This could explain the observed differences of HM if the galaxies in region B are meaningfully more distant than in region A.

Such an interpretation is rather doubtful because we have checked that there is no significant difference of the mean geometrical distances between regions A and B ( $L_B - L_A = 200 \text{ km s}^{-1}$ , in symbolic radial velocity), so that the observed velocity difference cannot be interpreted in this way ( $\langle V \rangle_B - \langle V \rangle_A = 2,412 \text{ km s}^{-1}$  for radio galaxies) and because Fig. 2 shows that differences of the mean HM exist within each narrow redshift band, so that the total average difference cannot be explained by a simple difference between the distribution of galaxies with distance in the two regions.

We must finally mention the possibility that the mean absolute magnitude of the observed galaxies is not constant over the sky and take into consideration possible uncertainties in the analysis. Contingent, however, on the verification of the effect with an improved sample, we tentatively propose two alternative interpretations:

(1) Light emitted by distant galaxies is redshifted when passing through clusters of galaxies (an effect which could be connected with 'tired light' theories<sup>17,18</sup>) or (2) distant sources are more luminous when seen through intermediate clusters of galaxies, which could act, for example, as gravitational lenses.

**Fig. 1** Distribution on the map of the sky of the clusters of galaxies and of our two samples of galaxies, in equatorial coordinates. The clusters are represented by their outlines ○, radio galaxies in region A; ●, region B. △, compact galaxies in region A; ▲, region B. The dashed line is the border according to Rubin *et al.*



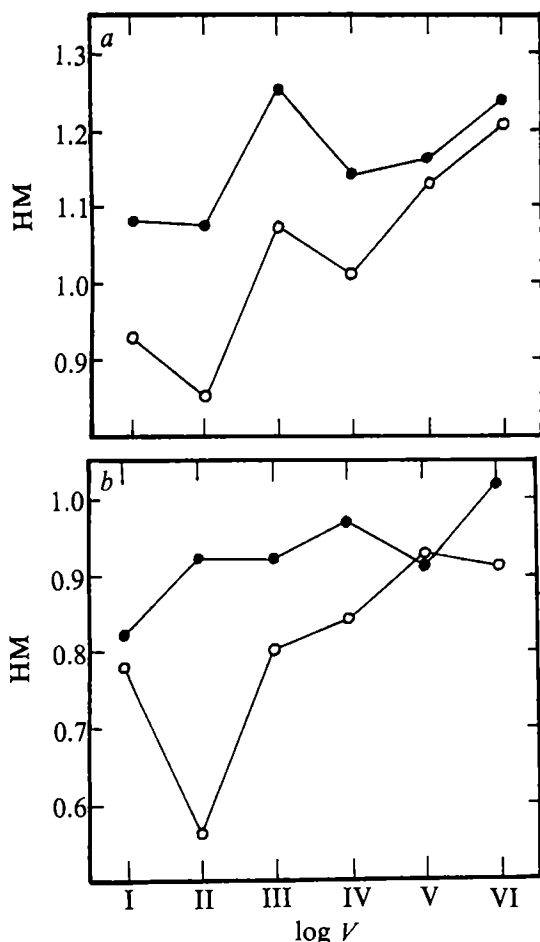


Fig. 2 ( $\log V$ , HM) diagram for (a) radio galaxies and (b) compact galaxies. The interval is divided into six narrow bands: I, 3.68–3.78; II, 3.78–3.88; III, 3.88–3.98; IV, 3.98–4.08; V, 4.08–4.18; VI, 4.18–4.30. In each band we calculate the mean of HM for region A (○) and region B (●). The difference between the two regions persists for each band.

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## Response of electrons in ionosphere and plasmasphere to magnetic storms

MEASUREMENTS of total electron content (TEC) using the Faraday polarisation-rotation and dispersive-group-delay techniques were carried out at Fort Monmouth, New Jersey<sup>1,2</sup> (40.18°N, 74.06°W) using v.h.f. and u.h.f. beacon signals emitted by the satellite ATS-6. A comparison of TEC values obtained by both these techniques yields the total electron content of the plasmasphere. During the severe magnetic storms of September 15–16 and September 18–19, 1974, rapid increases with sudden onsets were observed in TEC as well as in plasmaspheric content,  $N_p$ . These increases were followed by long periods of low values of TEC and  $N_p$ .

Figure 1 shows one such variation of  $N_F$  (ionospheric content from Faraday rotation measurements),  $N_T$  (total content from dispersive-group-delay measurements),  $N_p$  ( $\equiv N_T - N_F$ , total plasmaspheric content) and  $\tau$  ( $\equiv N_F/N_{max}$ , slab thickness, where  $N_{max}$  is the maximum electron density at Fort Belvoir, Virginia (37.9°N, 75.5°W) before and after a sudden commencement (SC). The  $K_p$  indices for that period are also indicated in the figure. While there was an abnormal evening increase in  $N_F$ ,  $N_T$ , and  $N_p$  between 1930 and 0230 UT on September 14–15, a period of relatively low magnetic activity, the response of  $N_F$ ,  $N_T$ ,  $N_p$  and  $\tau$  to SC is quite noticeable. For two hours after SC,  $N_F$ ,  $N_T$ , and  $N_p$  increased rapidly; they oscillated for the next three hours, and then decreased rapidly to values well below the monthly average. After SC,  $\tau$  also increased; it declined after 1930. Unfortunately, Wallops Island ionosonde data were not available for the period after ~2400, September 15. At the time of their maximum values (1545 UT, September 15),  $N_F$  and  $N_T$  were ~100% larger than their corresponding monthly averages. At the time of its maximum value (1730 UT, September 15)  $N_p$  was ~70% higher than its monthly average. During the period of depressed content values,  $N_F$  and  $N_T$  were at one point (1830 UT, September 16), ~45% of their monthly values, while  $N_p$  was ~21%.

Fig. 2 shows the variations of  $N_F$ ,  $N_T$ ,  $N_p$  and  $\tau$  before and after SC, which occurred at 1434 UT (nearly the same time SC occurred in the previous storm) on September 18, 1974. Before SC,  $N_F$  and  $N_T$  were slightly lower or nearly equal to their monthly averages, while  $N_p$  was considerably below its monthly average.  $\tau$  behaved similarly this time to its behaviour during the period preceding SC in the previous storm. For four hours after SC,  $N_F$  and  $N_T$  increased very rapidly; they then declined rapidly, and reached values below their monthly averages six hours after SC.  $N_F$  and  $N_T$  recovered quickly and attained values near their monthly averages. After 0700 UT on September 19,  $N_p$  increased rapidly after SC and reached its maximum ~15 min before that of  $N_F$  and  $N_T$ ; it then declined rapidly to values below its monthly average and remained there throughout September 19.  $N_p$  was not, however, as depressed this time as it was after SC in the days following the previous storm. For two hours after SC,  $\tau$  increased rapidly; it then decreased to values not significantly different from its pre-SC values before both storms. At the time of their maximum values (1830 UT),  $N_F$  and  $N_T$  were ~120% larger than their corresponding monthly means and  $N_p$  (at 1800 UT) was 67% larger than its monthly mean.

The average behaviour of  $N_F$  and  $\tau$  during geomagnetic storms at mid-latitudes has been discussed previously<sup>3</sup>. It was determined that  $N_F$  is characterised by an initial positive (a total ionisation enhancement relative to monthly mean values) phase lasting a few hours, followed by a negative phase (a total ionisation reduction) of a much longer duration, possibly lasting a few days. During the positive phase,  $\tau$  is also enhanced because the relative increases in  $N_F$  are greater than those in  $N_{max}$ . As indicated by Figs 1 and 2, the storms of September 15–16 and September 18–19 are not atypical, as their behaviour was similar to the average behaviour.

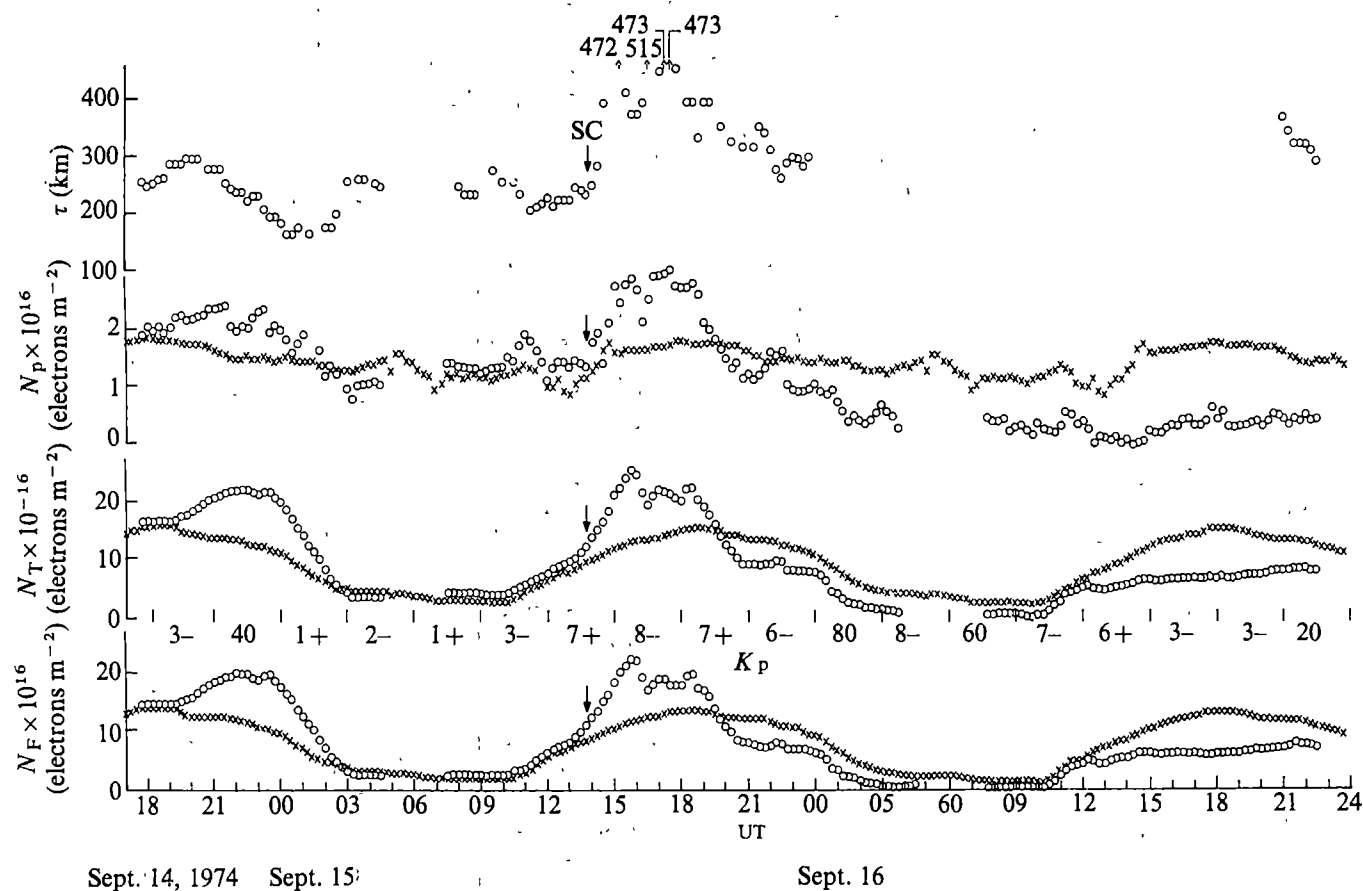


Fig. 1 Variation of  $N_F$ ,  $N_T$ ,  $N_P$ ,  $\tau$  and  $K_p$  from 1745 UT, September 14, 1974 to 2230 UT, September 16, 1974. Monthly averages of  $N_F$ ,  $N_T$ , and  $N_P$  are indicated by  $\times$ .

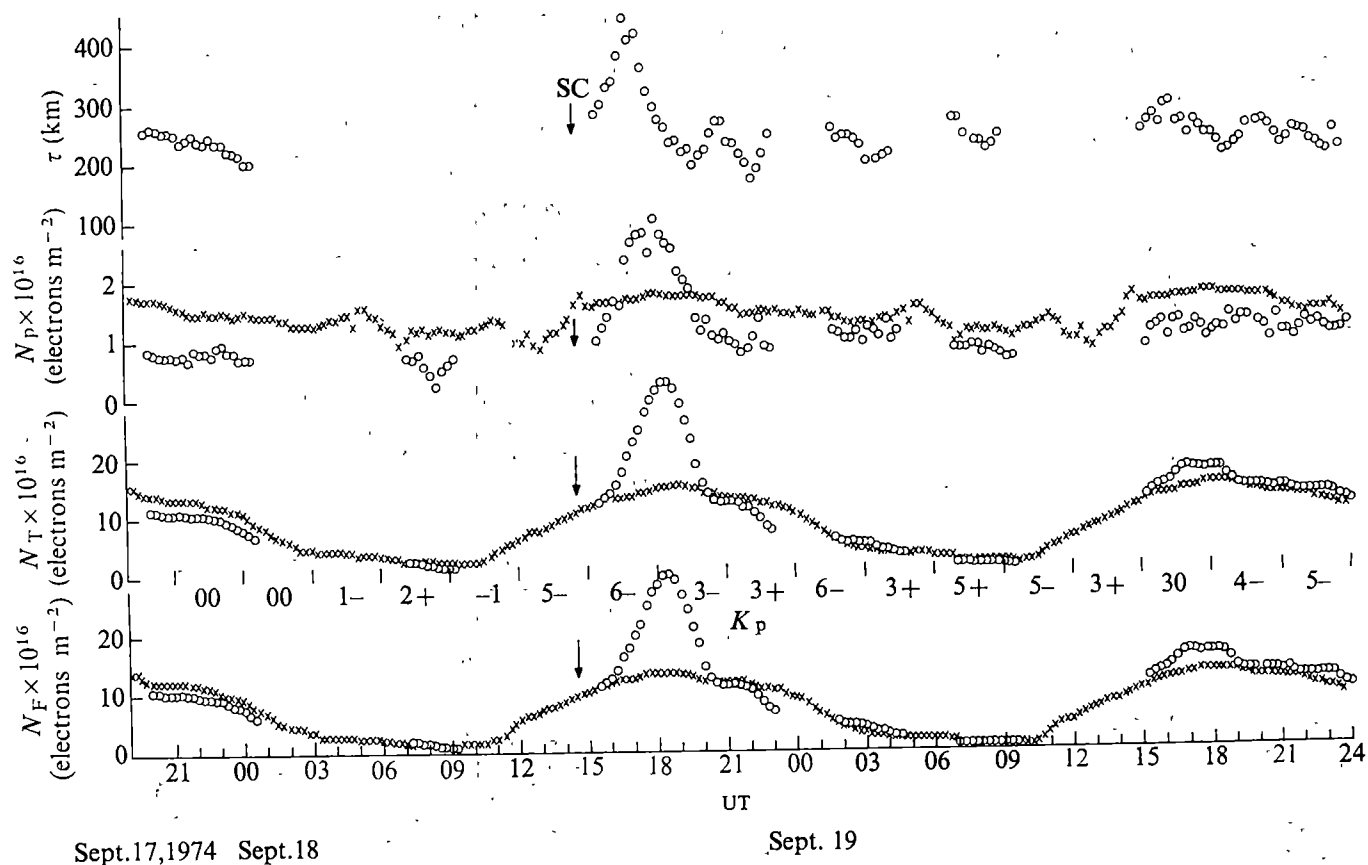


Fig. 2 Same as Fig. 1, but from 2000 UT, September 17 to 2400 UT, September 19, 1974.



The radio beacon experiment (RBE) of the ATS-6 yields new information regarding the behaviour of the plasmaspheric electron content,  $N_p$ , during magnetic storms. The RBE contains two relevant experimental modes. The first uses the Faraday rotation technique for determination of total electron content. Since the rotation is magnetic-field dependent, its value is weighted heavily in the vicinity of the Earth and it is assumed that the Faraday technique yields integrated content values ( $N_F$ ) to heights of  $\lesssim 1,500$  km. The second uses the dispersive-group-delay technique in which the phase of the modulation envelope between a carrier and its sideband is compared at two frequencies. Since the phase is insensitive to the Earth's magnetic field, this technique yields the number of electrons,  $N_T$ , along the entire path from the geostationary ATS-6 to the observer. The difference between the two, that is  $N_T - N_F$ , yields the total electron content above  $\sim 1,500$  km, which is referred to as the plasmaspheric content,  $N_p$ .

The increase in  $N_p$  following SC tends to support the theory that increases in the total electron content during the positive phase are caused, at least in part, by the lifting of the F layer to regions of reduced recombination. Apparently, the upward fluxes of electrons after SC cause  $N_T$  to increase faster than  $N_F$ . This means that some of the electrons which were counted by the Faraday rotation technique had moved from the ionosphere to the plasmasphere.  $N_T$  increased, since the diffusing electrons had reached regions of reduced recombination.  $N_F$  increased at a slower rate, since, while recombination was reduced, some electrons had moved to the plasmasphere and were no longer counted. The net result was enhancement of plasmaspheric content.

The negative phase of the magnetic storm, which commenced about five to six hours after SC, occurred simultaneously with  $N_F$ ,  $N_T$ , and  $N_p$  for both storms. As was previously suggested<sup>4</sup>, the heating effects of the neutral atmosphere, which disturb the normal production and loss processes in the ionosphere, were responsible for depletions of the ionospheric electron content. The plasmaspheric content, which is unaffected by normal production-loss processes, since these are ineffective at such heights, was very depressed compared to its corresponding mean monthly value. The plasmasphere is maintained by diffusive fluxes from the ionosphere. With the reduced values of the ionospheric densities, the plasmasphere was depleted by downward fluxes which seek to maintain hydrostatic equilibrium in the topside ionosphere.

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## Simultaneous small and large scale irregularities in the ionospheric F region

THE study of scintillation-producing irregularities over Australasia indicates a continuous spectrum of scale sizes of ionisation clouds<sup>1</sup>. Amplitude scintillations are most sensitive to small scale irregularities, peaking at 1 km transverse to the magnetic field<sup>1,2</sup>. The presence of large scale irregularities could be detected from the measurements of total electron content (TEC), using the Faraday rotation or dispersive-Doppler technique, on v.h.f./u.h.f. transmissions from satellites orbiting the Earth. There is considerable evidence of large wave structures in the F region; the wavelength and period of large ionospheric disturbances are similar to those of large scale gravity waves<sup>3,4</sup>.

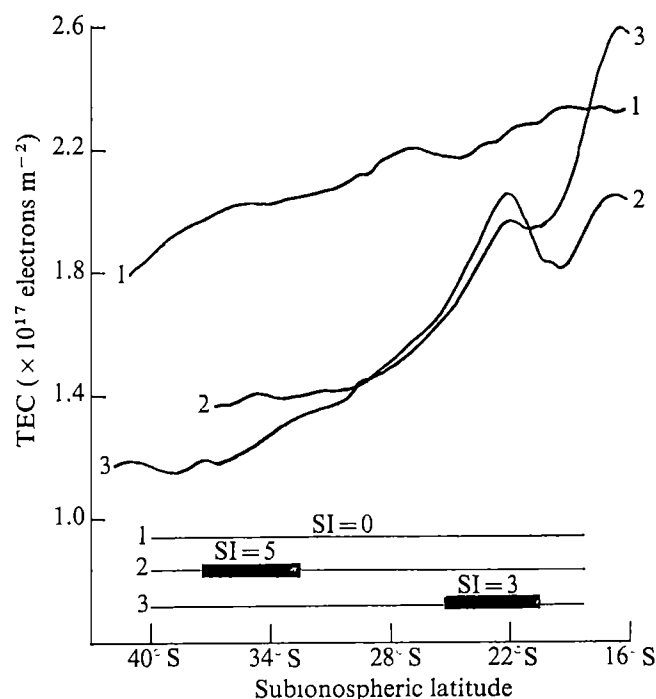


Fig. 1 An example of simultaneous occurrences of large fluctuations in TEC and scintillation activity.

Amplitude scintillation studies and TEC measurements have previously been conducted separately. This omission makes it difficult to cover the complete spectrum of irregularities since each of the techniques, used separately, has a limited spectral bandwidth. We therefore simultaneously recorded large and small scale ionisation structures in the F region. Amplitude scintillation studies were conducted using radio transmission from five US Navy navigational satellites (NNSS), at a frequency of 150 MHz. The recording station was in Brisbane (27°S, 152.9°E). Phase-locked receivers were used to receive radio signals from NNSS at two coherently related transmissions at frequencies 150 and 400 MHz. By employing a dual phase-locked receiver to monitor the phases of these two signals (and a suitable mixing technique to remove the vacuum Doppler) dispersive-Doppler phase shifts were measured. In turn, TEC computations were performed using the phase recordings. Thus, it was possible to record amplitude scintillations and TEC variations for the same satellite passes.

Figure 1 shows an example of simultaneous amplitude scintillations and TEC recordings obtained on November 24, 1973. Solid curves are TEC variations against sub-ionospheric latitude, assuming the irregularity height of 400 km above the Earth's surface. The corresponding scintillation regions are indicated by horizontal bars TEC and corresponding scintillations were recorded for NNSS passes with the following local times of the nearest approach: 1-1 (1756 LT), 2-2 (2046 LT) and 3-3 (2135 LT). The scintillation index (SI) varied from 60% (SI=3) to 100% (SI=5) modulation of the received signal (full definition of SI has been given elsewhere<sup>5</sup>). It can be seen that strong TEC variations were associated with pronounced scintillations during the second pass. The regions, where these two disturbances occurred were, however, largely different. A more typical situation is shown for the third pass when the large and small scale fluctuations occurred in a similar area.

We obtained 42 simultaneous TEC and amplitude records for November 1973 to January 1974. Forty per cent of the cases were recorded during quiet (SI=0) ionospheric conditions all with TEC amplitude (that is, percentage

deviation of TEC from the background ionisation) in the 0–5 range. During disturbed conditions ( $SI=1-5$ ), 5% of the recordings had TEC amplitude 0–5, 38% had amplitude 5–10 (average 7.2) and 17% had amplitude 10–15 (average 13.6).

Relatively large TEC fluctuations were always associated with scintillation activity, and there were very few scintillations whenever TEC amplitude was less than 5% of the background ionisation. The latitudinal occurrences of TEC fluctuations and simultaneous scintillations were also examined. There were 10 cases for which there was complete spatial coincidence, 9 cases of partial coincidence and 5 of no coincidence at all.

In general, TEC fluctuations had a larger horizontal extent than scintillations. They exhibited a quasi-periodic structure with the wavelength lying in the range 300–600 km.

It seems, from the above considerations, that there is good temporal correlation between the occurrence of large and small irregularities in the ionosphere. The spatial coincidence, however, of both structures is not complete. This may indicate that gravity waves, which are responsible for the quasi-periodic structure of TEC fluctuations, do not necessarily produce small scale irregularities. The simultaneous temporal occurrence of both structures seems to be due to a common source. It has been suggested that the source of ionisation clouds, responsible for mid-latitude scintillations, is located in the evening sector of the auroral zone<sup>3</sup>. There is also strong evidence that large scale gravity waves, observed at mid-latitudes, are produced in the evening sector of the auroral zone<sup>4</sup>. Thus, it is likely that there is a bulk motion of ionisation patches causing scintillations are wave motion associated with TEC fluctuations. Both structures once originated in the auroral zone seem to propagate towards the equator.

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## <sup>40</sup>Ar/<sup>39</sup>Ar dates for spreading rates in eastern Iceland

RADIOMETRIC dates for eastern Iceland have shown considerable scatter (see ref. 1 for a summary). This paper describes the results of the systematic dating of a stratigraphically continuous sequence of lavas (see Figure 1 for the sample locations; nomenclature follows ref. 2, from which further details can be obtained). As some of the lavas have experienced low-grade hydrothermal alteration<sup>3</sup> the <sup>40</sup>Ar/<sup>39</sup>Ar step-heating method was chosen, and the results of determinations on 18 samples from 11 sections are set out in Table 1.

Plateau ages are based on those steps whose apparent ages do not differ from the plateau mean by more than their estimated analytical error (s.d.). Means were calculated in two ways: weighting by the fraction of <sup>39</sup>Ar released during the step, and the inverse of the variance. As the difference in most cases was very small (suggesting the two methods are not independent) ages calculated using the latter method have been used in subsequent parts of this paper as it is the one in common use. All plateaux had at least four steps and comprised > 50% of

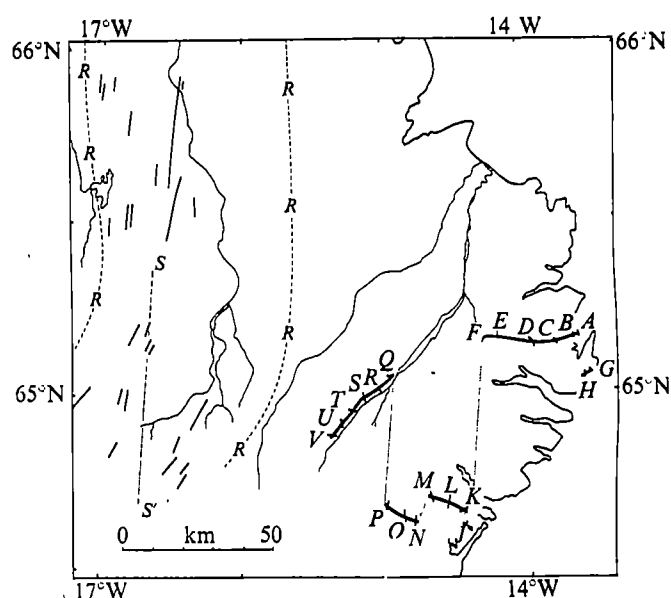


Fig. 1 Map of eastern Iceland. The large letters show the locations of the sections sampled by Dagley *et al.*<sup>2</sup>, from whose collection the dating samples were selected. The dashed lines  $R$  denote the limits of the present active zone, and the heavy lines within this zone signify post-glacial fissure eruptions. The line  $S-S'$  approximates the centre of the active zone for the sections  $A-V$ . Map after ref. 2 and 15.

the <sup>39</sup>Ar released. Random deviations from the plateau occurred at both the lowest and highest temperature steps. In the case of K9 the initial step, involving 21% of <sup>39</sup>Ar, gave an apparent age of  $143 \pm 16$  Myr, and though steps 2–5 gave a convincing plateau, this plateau age is significantly higher than that of K36 and appears anomalous in the succession; it therefore seems likely that some excess argon has been retained at all temperatures. As a saddle-shaped curve is a common feature of samples containing excess argon, of which the lowest part is sometimes horizontal enough to be considered a plateau<sup>4,5</sup>, there is therefore the possibility that other samples which gave a high age for the initial step have a systematically high plateau age, but as the initial step of no other sample was so high or comprised so much <sup>39</sup>Ar, the error is considered negligible.

Fig. 2 Possible flow profiles. Three curves of the form of equation (2) for different values of  $k$  are given. Also shown are three observed horizons indicated by symbols: circles, Bodvarsson and Walker<sup>6</sup> Fig. 3, section  $BB'$ , profile between horizons H.T. and Gr.P; + and × respectively from right and left horizons of Fig. 2, Walker<sup>3</sup>. Curve  $A$  is assumed steepest flow profile (stratigraphic horizon) used to give probable minimum correction. Inset: Idealised section through lava pile. The source is to the left; the vertical scale is exaggerated.

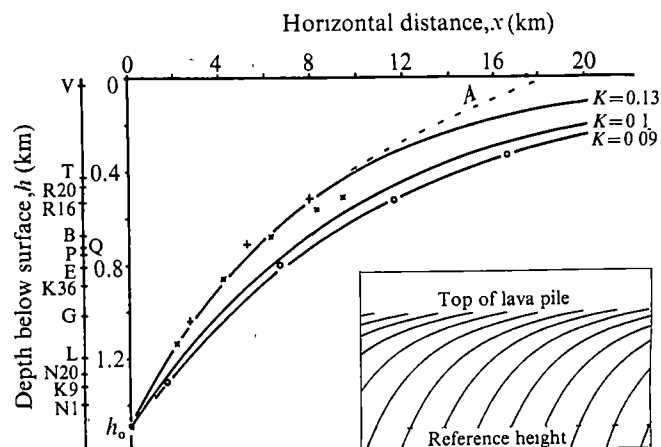


Table 1 Results of step-heating samples of lava

Section	Lava and core*	No. of plateau steps	% $^{39}\text{Ar}$ on plateau	Mean age I (Myr)†	Mean age II (Myr)‡	Total fusion age (Myr)§
V	18-1	4	85	2.0	1.96±0.44	
T	17A-2	5	100	3.1	3.31±0.72	3.1±0.5
R	20A-2	5	82	4.3	4.33±0.62	3.7±0.8
	16-2	4	77	5.3	5.26±0.15	6.4±0.4
Q	13-1	4	90	5.8	5.81±0.16	6.1±0.6
P	14-1	5	80	5.7	5.68±0.17	
N	20-2	9	99	6.5	6.48±0.73	6.2±0.9
	1-1	4	60	7.8	7.83±0.11	7.0±0.3
L	33C-1	5	92	8.1	8.19±0.72	8.6±0.5
K	36A-2	5	86	9.6	9.58±0.41	9.5±0.5
	36A-2	6	91	10.2	10.11±0.27	10.4±0.4
	36A-1	5	87	10.2	10.18±0.22	10.6±0.4
	9-1	4	77	11.6	11.79±0.40	40.0±5.0
E	14-2	5	55	9.8	9.77±0.33	7.0±1.5
B	14-2	7	80	12.0	12.30±0.79	
	19-1	6	95	11.6	11.98±0.78	
G	19-1	4	80	13.2	13.19±0.10	
	21-1	4	75	12.9	12.92±0.14	

\* Samples are arranged in stratigraphic order.

† Weighted by %  $^{39}\text{Ar}$  released in each plateau step.

‡ Weighted by inverse of variance of each step age.

|| Deduced by summing all steps.

§ Estimated, as lowest temperature step not measured.

With this exception, the stratigraphic consistency of the dates shows the validity of the method.

Table 1 (and Fig. 3) shows that there can be no major time-gaps in the succession, and therefore affords no support for the large westward displacement of the spreading zone, postulated by Ward, which would result in a hiatus between sections P and Q<sup>6</sup>. Nor has a hiatus been found in the Western Iceland succession<sup>8</sup>. Table 1 also shows that evidence for glaciation is found in sections as old as P<sup>2</sup>, ~ 5.5 Myr ago.

Calculations of spreading rates are not possible until allowance has been made for the fact that as a flow (or the group of flows which constitutes a section) has considerable lateral extent, it may be sampled over a range of distances from the spreading zone. In eastern Iceland the lava groups, on average, increase both their dip and thickness in a regular manner as they are followed westward<sup>9</sup>, so that, in principle, the distance of a flow from the axis of the spreading zone can be defined uniquely, though somewhat arbitrarily, by measuring it at some specified reference height within the lava pile (Fig. 2, inset). If a sample was collected at a different height, its distance from the spreading axis can be corrected to that height, provided the cross-sectional shape of a lava flow ('horizon') is known. In practice there are complications; the lava pile has been distorted, there are internal irregularities and erosion has removed its top. Thus, to make the correction it is necessary to know the position of the sample in the original pile, as well as the shape of the average horizon, both of which have to be deduced indirectly.

The height of the original top of the pile can be inferred from a study of the dyke swarms and the zeolite grade metamorphism, and the two methods give consistent results<sup>3,9</sup>. A map showing the height of the original surface (taken as 600 m above the top of the analcite zone) has been published<sup>10</sup>; it varies from 0.7 to 1.7 km above present sea level.

The deduction of the shape of unmapped parts of the pile is not so easy, but depends upon the regularity of profile seen in exposed sections<sup>9</sup>. If it is assumed that a group of lavas thins away from the spreading zone according to

$$z = z_0 \exp(-kx) \quad (1)$$

where  $z$  and  $z_0$  are the thicknesses of the group at two places separated by a distance  $x$  (measured horizontally perpendicular to the spreading zone), and if it is further assumed that the flows of the pile have the same profile over a significant distance

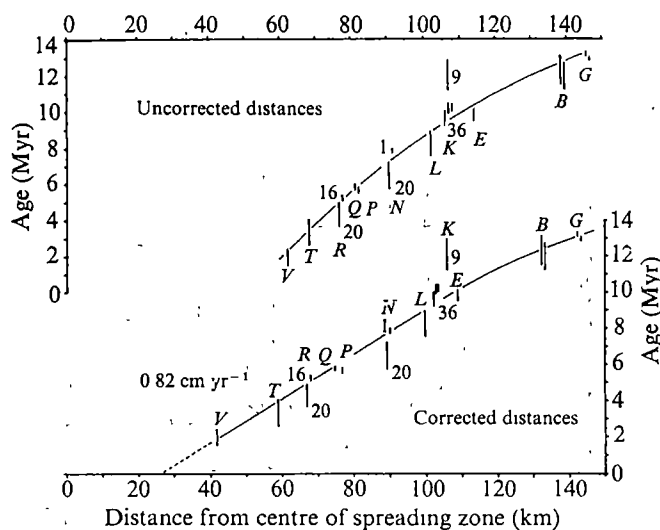
(Fig. 2, inset), then it necessarily follows that the horizons have the form

$$h = h_0 \exp(-kx) \quad (2)$$

where  $h_0$  is an arbitrary depth that sets the zero of  $x$  (Fig. 2).

$k$  can be estimated from reports in the literature in a number of ways; the rate at which the dip decreases in a vertical traverse of the pile  $[(d/dh)(dh/dx)]$  is simply  $-k$ . Dagley *et al.*<sup>2</sup> state that "there is a regular upward decrease in the dip of a lava flow averaging 1° per 170 m increase in altitude", which, since it does not depend upon the altitude, supports equation (1), and yields  $k = 0.102$ . A second method depends on the dip observed at a specified height in the pile: that at sea level near section A (estimated at about 1.3 km below the original surface) is given as 6–8° (ref. 2). If a mean of 7° is chosen, equation (2)

Fig. 3 Age of samples against distance from spreading ridge. Distances have been measured perpendicularly to the line S-S' of Fig. 1; in the upper part these distances have been used unaltered, but in the lower part they have been corrected to a reference height 1.5 km below the surface of the pile, according to curve A of Fig. 2. Errors in distance are unknown but could be large. Samples are identified by section letter, plus number where necessary.



gives  $k = 0.103$ . Figure 2 shows a number of curves obeying equation (2) with  $h_0 = 1.5$  km and different values of  $k$ . Also shown are some horizons described elsewhere (approximately perpendicular to the spreading axis), after correction to the inferred depth below the original surface. The actual profiles only approximate the curves from equation (2), and even then have a range of values of  $k$  from roughly 0.09 to 0.13. In addition, it should be noted that the mathematical model must breakdown as the profile approaches the surface, for the theoretical curves give some lavas extending to infinity, as implied in equation (1). Curve A of Fig. 2, an assumed extrapolation of the steepest of the three observed horizons, has therefore been used to make corrections, which are probably minimal.

Figure 2 is used to correct the sample positions to a common depth, taken as 1.5 km (the more obvious choice of the surface as reference height is precluded by the inadequacy of the model). The effect of the correction is most marked for the youngest and nearest-surface sections ( $R-V$ ), and results in an increase in the apparent spreading rate over the time spanned by these sections. The errors on the largest corrections are more than proportionately large, as they depend on the estimation of the sample position relative to the original surface, the orientation of the line  $S-S'$  and on the shape of the flow horizon near the surface, where the model breaks down and where field observation is lacking. The correction for sample V18 is, however, unlikely to be  $< 18$  km and is probably more, so that the spreading rate for the period from 10–2 Myr was probably uniform and not less than  $0.8 \text{ cm yr}^{-1}$  (half rate). At the present level of accuracy this is not distinguishable from the spreading rates on the Kolbeinsey Ridge to the north of Iceland ( $0.82 \text{ cm yr}^{-1}$  on the eastern side<sup>11</sup>) or the Reykjanes Ridge to the south [ $\sim 1 \text{ cm yr}^{-1}$  (ref. 12)].

The corrected part of Fig. 3 extrapolates, for zero age, to a position about 30 km east of the centre of the present active zone, but within the active zone. This figure would be reduced if larger corrections were applied; on the other hand, if the distance from the spreading axis were referred to a reference height nearer the surface the figure would be increased. Before this intercept can be interpreted, a detailed understanding of the zone in which the lava pile is formed is needed; suggested models involve an active width of 50 km (ref. 13) or 70 km (ref. 14), and therefore, considering the large possible errors in Fig. 3, no significance is attached at present to the non-zero intercept.

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## Direct conversion of graphite to diamond under static pressure

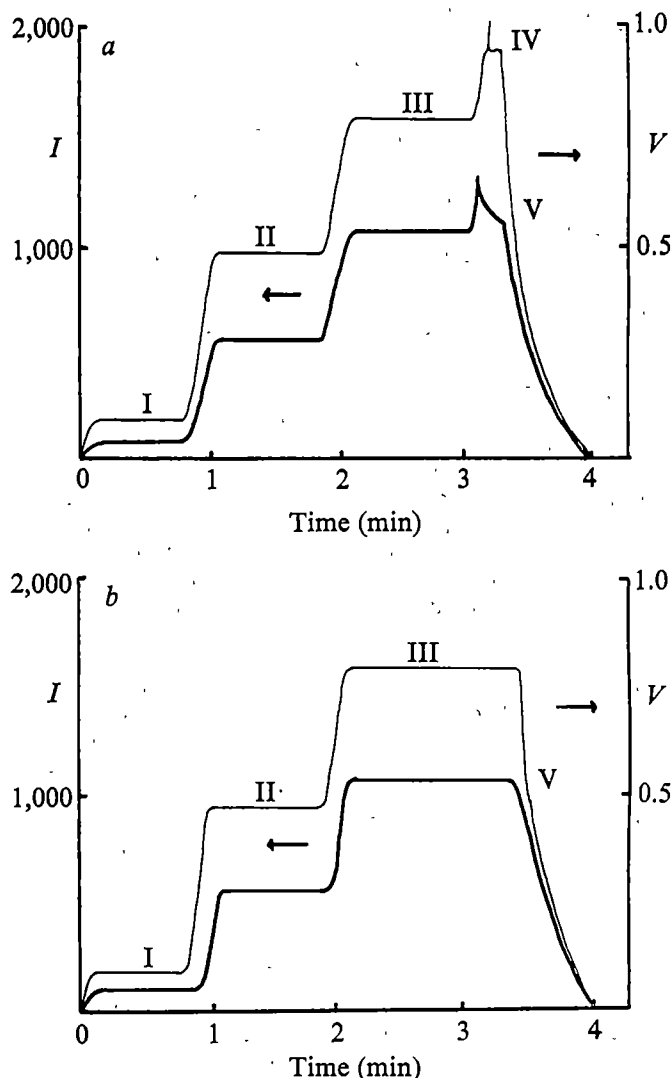
WE report the preparation of cubic type diamonds, by heating a graphite bar by passing an electric current directly through the specimen; we used a modification of the techniques developed in refs 1–4.

We obtained more than 60% of the theoretical yield. The transparent diamonds obtained were in the aggregated state, with a size of about 0.1 mm. The aggregate was found to be composed of a number of crystals with size of  $\sim 10$ – $20 \mu\text{m}$ , which is larger than the size (200–500 Å) of diamond crystals synthesised by flash heating<sup>5</sup>.

A spectroscopic grade graphite bar, 3 mm in diameter and 7 mm in length (about 80 mg), was used. The specimen was subjected to a pressure of  $140 \pm 10$  kbar, and then an electric current was passed through it. Figure 1 shows a plot of current–voltage against time: *a* is a typical case where diamonds were successfully obtained, *b* is a case where diamonds were not obtained.

The voltage was increased at fixed intervals and until step III, Ohm's Law was obeyed. When the voltage was increased to 0.95 V at step IV, a sudden increase in voltage and a decrease in the electric current were observed. The

Fig. 1 *a* and *b*, Current and voltage against time for spectroscopic graphite bar at 140 kbar. *a*, Voltage taken up to 0.95 V; *b*, voltage taken to 0.8 V.



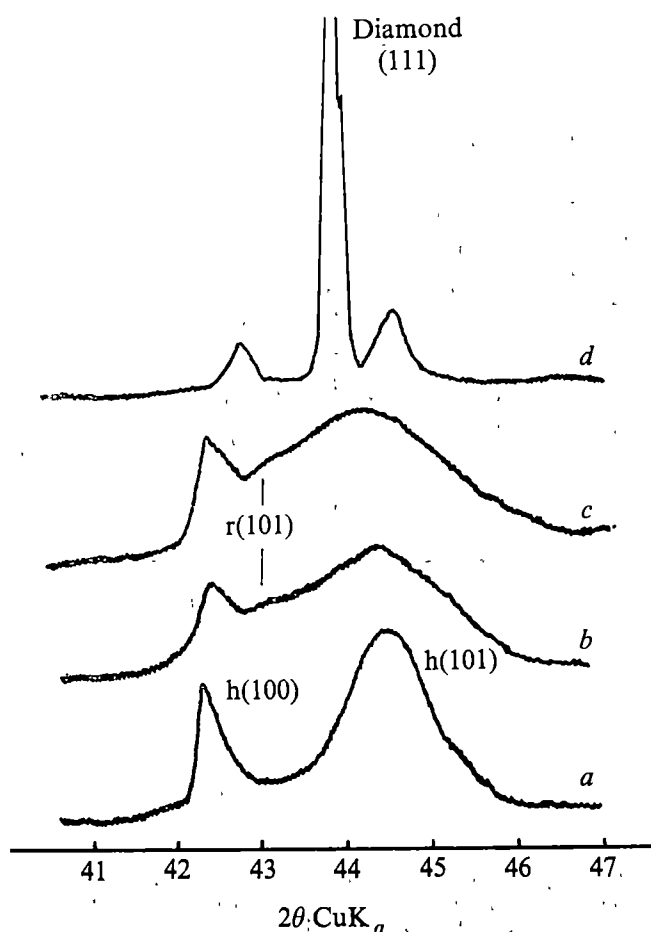


Fig. 2 X-ray powder diffraction patterns of the original graphite and the heat-treated specimens. *a*, Original graphite; *b*,  $I, V = 0$  and  $P = 140$  kbar; *c*, specimen obtained from the experiment recorded in Fig. 1*b*; *d*, specimen obtained from the experiment recorded in Fig. 1*a*.

voltage was then maintained at a constant 0.95 V, and the electric current decreased gradually for about 20 s. This phenomenon was similar to the observations of Bundy<sup>1</sup>, but the conversion time of graphite to diamond in his experiment was said to be of 'millisecond' order, whereas the corresponding time in our experiments seemed to be of the order of seconds. Using the flash heating technique, homogeneous heating of the specimen should be obtained, with consequent rapid crystal transformation whereas using our resistance heating technique, a radial temperature gradient should be created. The core of the specimen would then be the first part to turn into diamond, and the transformation would subsequently propagate to the surface.

At step V, the heating power was purposely decreased. This was necessary to prevent the melting of the pressure medium, and contamination of the diamond.

If the voltage was increased to only 0.8 V, as shown in Fig. 1*b*, the transformation of graphite to diamond did not occur. The temperature at step IV in Fig. 1*a* may, therefore, be estimated, from the reaction diagram of Bundy<sup>3</sup>, to be 3,000–3,500 K.

The X-ray powder diffraction patterns of the original graphite specimen and the heat-treated specimens are shown in Fig. 2. By pressurising the original graphite, the hexagonal lattice of the graphite was distorted and partly converted to the rhombohedral lattice. Rhombohedral graphite was also obtained by grinding<sup>6</sup>. The rhombohedral lattice is stable on heating up to stage III and it was therefore concluded that the transformation to diamond in the metastable region of graphite must proceed through

a drastic change in the chemical bond. DeCarili and Jamison have also proposed that diamond is formed from the rhombohedral lattice of graphite<sup>7</sup>. Establishment of the mechanism of the transformation process will need, however, more detailed physicochemical experiments.

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## Language perception of 2-month-old infants shows effects of both innate mechanisms and experience

VOICING differences distinguish two or more stop consonants in nearly all the world's languages. It has been found<sup>1</sup> that voicing distinctions for stops in word-initial position can usually be characterised by differences in voicing onset time (VOT), where VOT is defined as the time between the stop release burst and the onset of vocal cord vibration (voicing). For example, the English phonemes /p/ and /b/ differ in this way; voicing follows release in /p/, while voicing is approximately simultaneous with release in /b/.

Eimas *et al.*<sup>2</sup> found that American infants as young as 1 month old discriminated between the synthetically produced speech sounds, [b] and [p]. These infants failed, however, to discriminate between speech sounds which were separated by the same VOT difference as [b] and [p], but were members of the same phonemic category in English. (This result has been replicated for stimulus separations as small as 20 ms VOT and as large as 100 ms VOT<sup>3</sup>.) Thus, the infants, who do not yet produce these sounds, showed the same discrimination pattern as American adults; discrimination between phoneme categories was better than discrimination within phoneme categories<sup>4</sup>. Consequently, it has been proposed that the perception of voicing may be accomplished by innate perceptual mechanisms specifically tuned to perceive speech. To determine whether the discrimination of the English voicing distinction is independent of earlier linguistic exposure, I have investigated the discrimination of voicing onset by infants reared in a linguistic environment in which the English voiced/voiceless distinction is not used.

Kikuyu, a Bantu language spoken in Kenya, has only one labial stop, a prevoiced /b/, in which voicing substantially precedes articulatory release by an average value of 64 ms. The language distinguishes between prevoiced and voiced stops for other places of articulation, but Kikuyu has no stops in which voicing onset is substantially delayed relative to stop release as in the English voiceless, /p/, /t/, and /k/.

Discrimination was tested by a conditioned non-nutritive sucking habituation technique<sup>5</sup>. The infant sucked a blind nipple, which was attached to a pressure transducer. Criterial sucks whose amplitude exceeded an individually adjusted threshold (the baseline rate was approximately between 15 and 30 sucks min<sup>-1</sup>) activated a counter and resulted in 1 s. of sound presentation. The sound source was a tape loop with alternating syllables such as [ba] and silent intervals each 500



ms in duration. The sound source was gated in and out by a variable resistance device to minimise clicks. One or more criterial sucks  $s^{-1}$  continuously maintained the auditory signal at a comfortable listening level that produced few startle responses.

Typically, the infant's sucking rate increased over the baseline rate after a few min of pairing sucking and sound, presumably because of the reinforcing properties of the sound; eventually, however, the sucking rate decreased. This decrease has been attributed to habituation to the sound or its reinforcing value<sup>2</sup>. When the sucking rate had decreased by  $\geq 20\%$  of the rate in the immediately preceding minute for two consecutive minutes or had decreased by  $\geq 20\%$ ,  $\geq 0\%$ , and  $\geq 20\%$  respectively in three successive minutes, the sound was changed without interruption. The experiment was terminated 4 min after the sound shift. The rationale for this procedure is that if the sucking rate increases after the sound shift relative to a control condition with no sound shift, the sound shift must have been discriminated.

All stimuli were generated on the Haskins Laboratories' parallel resonance synthesiser and had the same formant frequencies and amplitudes as the stimuli used by Eimas *et al.* In all there were six syllables, a labial stop followed by the vowel [a] with VOTs of: -30 (prevoiced), 0 (voiced), 10 (voiced), 40 (voiceless), 50 (voiceless), and 80 (voiceless), which would be identified by English speakers as /ba/, /ba/, /ba/, /pa/, /pa/, and /pa/ respectively.

There were three experimental conditions with the following VOTs: (-30, 0), (10, 40), (50, 80) and a control condition in which pre- and post-shift sounds were the same, distributed equally over the six VOT values. The order of the three experimental conditions was counterbalanced across subjects as was the order of sound presentation within each condition. The control condition was either first or last. Each infant participated in all conditions.

Thirty-six periurban Kikuyu infants with a mean age of 63 d (s.d. = 12 d) participated in the study. All infants had heard only Kikuyu spoken in the home.

As in previous investigations<sup>6</sup> using this technique with American infants, data were excluded for subjects who did not demonstrate adequate conditioning or habituation. Data were excluded if: (1) the pre-sound shift conditioning and habituation period was less than 4 min long and/or (2) if there were fewer than 15 sucks in either of the 2 min directly preceding the sound shift. The reason for the second criterion was that the infant had habituated too much, and consequently the stimulus would not be presented often enough to observe changes in its effect.

Analysis (unweighted means solution, repeated measures on two factors) of sucking rate in the 4 min directly preceding the sound shift indicated that all conditions were similar before the sound shift. (An analysis of variance revealed no significant effects of conditions or a conditions-minutes interaction.)

Discrimination was measured as an increase in sucking rate during the 2 min directly after the sound shift compared with the 2 min immediately preceding the shift. Analyses were performed using these difference scores. The analyses were *t*-tests, using split-significance levels ( $\alpha = 0.05/k$ , where  $k = 3$ ). The analysis of these reduced data required the assumption of zero main effects and interactions for subjects. Since an analysis of the full data set showed that the between subject differences were near zero, this seemed to be a reasonable assumption. Both the (10, 40) and (-30, 0) conditions were different from the control condition ( $P < 0.05$ ). The (50, 80) condition was not, however, different from the control condition; nor were there any significant differences among the three experimental conditions. Figure 1 shows the mean change in response rate for all four conditions.

Kikuyu infants thus made two linguistically relevant discriminations; they discriminated a [p] in which voicing preceded release from one produced with simultaneity of voicing and release, and they also discriminated a [b] produced with approx-

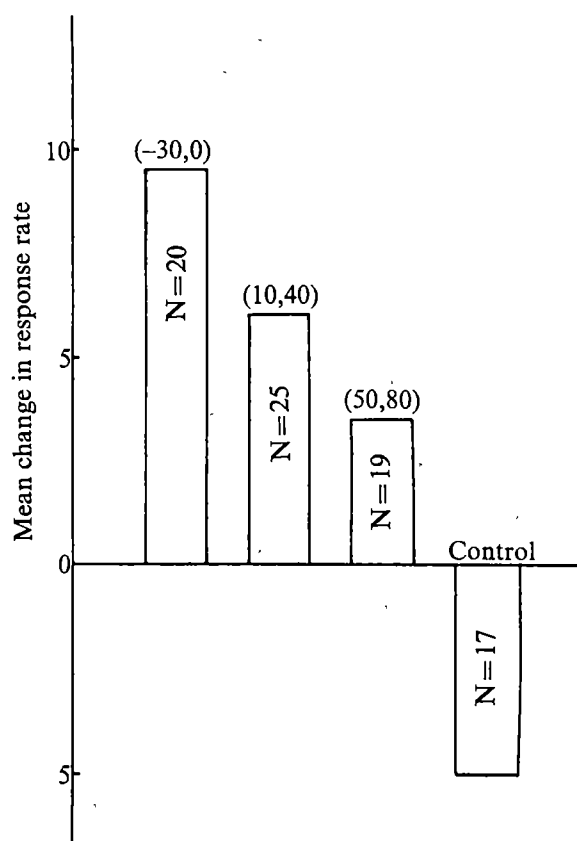


Fig. 1 Mean change in response rate per min as a function of experimental condition. The s. e. m. for the four conditions are: (-30,0) 3.24, (10,40) 2.77, (50,80) 3.58, and (control) 4.32.

imately simultaneous onset of voicing and release from a labial stop produced with substantial voicing lag [p]. It should be noted that adult Kikuyus showed the same discrimination pattern as the infants<sup>7</sup>. Thus, the ability to discriminate the voiced/voiceless distinction is not lost in the absence of relevant linguistic exposure, suggesting that this voicing distinction due to its psycho-acoustic properties is naturally discriminable.

The results of the present study differ from those of Eimas *et al.*, who found no reliable discrimination of the prevoiced/voiced distinction in American infants. Eimas<sup>3</sup> reports that American infants discriminated the prevoiced/voiced distinction. The stimulus separation used (70 ms) was, however, much larger than the one used here, and was of the order of three times larger than required for adult phoneme discrimination in any language that has been studied.

The differences between the present study and that of Eimas *et al.* seem to be attributable to differences in American and Kikuyu linguistic environments. Kikuyu infants hear only one labial stop, a prevoiced /b/. But they do hear prevoiced stops contrasted with their voiced counterparts for other places of articulation. Thus, they are exposed to the acoustic cue or cues underlying the prevoiced/voiced distinction. On the other hand, American infants do not hear prevoiced stops in their linguistic environment. Thus, the results suggest that previous exposure to the prevoiced/voiced distinction in the language or perhaps exposure to the acoustic cues underlying this distinction is a prerequisite for discrimination of the contrast. On the other hand, the voiced/voiceless distinction can be made in the absence of relevant linguistic exposure, since Kikuyu infants do not hear this voicing distinction used in their language. Thus, the results suggest that there is an interaction between nature and nurture; some phonetic or acoustic discriminations may be universal, whereas others seem to require previous relevant exposure.

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## Behavioural fever in teleost fishes

THE ectothermic lizard *Dipsosaurus dorsalis* has been shown to exhibit a behavioural fever<sup>1</sup> when injected with killed *Aeromonas hydrophila*, a Gram-negative bacterium pathogenic to mammals, reptiles, amphibians and fishes, causing haemorrhagic septicaemia<sup>2,3</sup>. The behavioural fever in lizards has been shown to have survival value<sup>4</sup>.

Mammals and birds use behavioural as well as physiological means to increase body temperature during a fever<sup>5,6</sup>, whereas ectotherms such as lizards and fishes are limited largely to behavioural means alone<sup>1,7</sup>. Evidence is accumulating that the hypothalamus controls thermoregulatory responses (both behavioural and physiological) in all classes of vertebrates, including fishes<sup>1,7-11</sup>. Drugs such as pentobarbitol which alter thermoregulation in mammals by acting on the hypothalamus<sup>12</sup> also affect behavioural thermoregulation in fishes<sup>13,14</sup>.

Fever results from the effect of pyrogens on the hypothalamus<sup>15,16</sup> by increasing the set points of the thermostat control centre<sup>1</sup>. Vaughn *et al.*<sup>1</sup> speculated that the behavioural component of fever might have evolved in early reptiles or even amphibians, and presented evidence for this in reptiles. We report here evidence that a similar behavioural febrile response exists also in fishes.

We tested eight bluegill sunfish, *Lepomis macrochirus* Rafinesque (120-155 mm standard length), and two large-mouth blackbass, *Micropterus salmoides* (Lacépède) (110-160 mm s.l.), matching the sample size of ten animals tested by Vaughn *et al.*<sup>1</sup>, plus an equal number of controls. The normal thermoregulatory behaviour of these species has been extensively studied<sup>17-21</sup>.

We allowed each fish to thermoregulate in a device which enables a fish to control water temperatures, and thus body temperatures, by its movements between two chambers of different temperatures controlled by photo-transistor relays which monitor the position of the fish. The operation of this device has been described previously<sup>17,18</sup>, having been based on an earlier design<sup>20-22</sup> which is similar in principle to that used by Vaughn *et al.*<sup>1</sup> for lizards. Recent work in our laboratory has shown that mean deep body temperatures in our device do not differ significantly from mean occupied temperatures, nor from mean water temperatures.

The fish were maintained in aquaria at 22-24°C, on a light-dark (LD) 12:12 photoperiod. Fish were tested individually. Each fish was allowed an introductory period of 24 h in which to reach its preferred final temperature (~30°C) and become acclimatised to it<sup>17</sup>. Then, mean occupied temperatures were monitored for a control period of 24 h. The fish was then injected intraperitoneally with 0.75 cm<sup>3</sup> of fish physiological saline containing 4 × 10<sup>8</sup> killed *A. hydrophila* cells, prepared as described by Vaughn *et al.*<sup>1</sup>, and thermoregulatory performance was monitored

Table 1 Mean preferred temperatures (±1 s.e.) before and after injection of *A. hydrophila* in ten fish

Species	s.l. (mm)	°C before injection	°C after injection	+ΔT (°C)
<i>Micropterus salmoides</i>	110	29.6±0.3	31.9±0.2	2.3
<i>Micropterus salmoides</i>	160	30.6±0.3	32.3±0.3	1.8
Grand mean (bass)		30.1±0.5	32.2±0.3	2.1
<i>Lepomis macrochirus</i>	120	31.9±0.2	33.4±0.2	1.5
<i>Lepomis macrochirus</i>	125	30.9±0.2	32.4±0.3	1.5
<i>Lepomis macrochirus</i>	130	31.6±0.3	34.4±0.3	2.8
<i>Lepomis macrochirus</i>	130	32.5±0.2	37.2±0.2	4.7
<i>Lepomis macrochirus</i>	145	27.9±0.3	30.4±0.2	2.5
<i>Lepomis macrochirus</i>	150	29.3±0.3	32.2±0.2	2.9
<i>Lepomis macrochirus</i>	150	30.8±0.2	33.8±0.3	3.0
<i>Lepomis macrochirus</i>	155	29.2±0.2	31.4±0.2	2.2
Grand mean (bluegill)		30.5±0.6	33.2±0.7	2.7
Grand mean (all fish)		30.4±0.5	33.0±0.6	2.6

for a further 24 h. An equal number of matched control fish were similarly handled, and injected only with fish physiological saline.

Hourly occupied temperatures were tabulated for the 24-hour periods before and after injection of each fish. The mean occupied temperatures were calculated, as were grand means (means of means) for each species and for all the test fish (Table 1). All bacteria-injected fish showed a significant increase (*t* test, 0.05) in preferred temperature: mean temperature increase (ΔT) was 2.6°C for all test fish, 2.7°C for the bluegills and 2.1°C for the bass. The controls showed no increase.

We conclude that fish exhibit a behavioural fever, as do lizards, under the influence of bacterial pyrogens. This is probably due to a direct influence on the hypothalamic thermoregulatory centre, although direct evidence for this mechanism in fish awaits further neurophysiological study. Our data support the hypothesis that the neural mechanism for the control of thermoregulation, in the preoptic hypothalamus, had a common origin early in the evolutionary history of vertebrates<sup>1,7-11</sup>. Fever as an adaptive response to infection<sup>2-4</sup> may have similarly had its origin early in vertebrate history, even before the evolution of a terrestrial mode of life.

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## Gravity as the sole navigational aid to the newborn quokka

ALTHOUGH there is general agreement on the sequence of events at birth for several marsupial species<sup>1</sup>, it is still not known how the newborn joey navigates on its journey from the mother's cloaca to her pouch. Frith and Calaby<sup>2</sup> have postulated that, at birth, the red kangaroo (*Megaleia rufa*) "has large nostrils and presumably a well developed sense of smell which probably plays a major part in its location of the pouch". On the other hand, Hartman<sup>3</sup> has suggested that the newborn opossum (*Didelphis virginiana*) was "negatively geotropic" though he has now modified this view considerably<sup>4</sup>, as histological studies have revealed that the vestibular apparatus of the newborn animal is undifferentiated and therefore non-functional<sup>5,6</sup>. To investigate whether one of these mechanisms<sup>2,4</sup> could account for the behaviour of the newborn quokka (*Setonix brachyurus*) we have observed parturition in this macropodid marsupial.

Female quokkas mate soon after giving birth and such animals often develop a blastocyst in the state of diapause in the uterus. Removal of the pouch young causes this blastocyst to resume development so that a birth occurs about 26 days later<sup>7</sup>. In March 1974 and 1975 pouch young were removed from a total of 14 quokkas obtained from a wild population on Rottnest Island, Western Australia. The animals were transferred to a controlled environment 22 d later, then observed continuously. In all, seven animals gave birth; four births were seen and one was recorded in detail on videotape.

Our observations of these births confirm those of Tyndale-Biscoe<sup>8</sup>. In spite of intensive antepartum grooming of the pouch and cloacal region, there is no evidence of a path made by the mother to facilitate the passage of the joey from the cloaca to the pouch. The mother's position during birth was such that the abdominal wall was almost vertical and the lower lip of the pouch pouted outwards. Observation of the actual entry of the joey into the pouch was difficult because of the stance of the mother but on one occasion the joey seemed merely to tumble in over the lip.

After a birth in 1974 the joey was retrieved as soon as it had reached the pouch. The mother was then held vertically, head up, and the joey was placed on the upper (anterior) lip of the pouch. During the next 3 min it climbed about 3 cm upwards away from the pouch. The mother was then turned upside down. The joey faltered and eventually turned completely around so that its head was again pointed upwards, towards the pouch. It then became entangled in the mother's fur and made no progress. When the mother was returned to the head up position the joey reversed its direction again and progressed away from the pouch. It was then removed from the mother's chest and replaced near the cloaca, whence it succeeded in making a second journey to the pouch.

On the occasion of the videotaped birth in 1975 the mother was removed from her cage and held almost vertical, with her right side slightly lower than her left, while the newborn joey was still making its initial journey to the pouch. This led to its direction changing slightly so that it passed by the pouch; it did not falter but continued to climb to the mother's chest, leaving a distinct path, about 1 cm deep, in the fur. The mother was then turned to the head down position and, as before, the joey slowly turned around, this time without becoming entangled in the fur,

and made progress back towards the pouch. The joey was not handled during this experiment.

These observations suggested that the newborn quokka has a righting reflex; we found that it could also turn over when placed on its back. But older pouch young, up to the age of 65-70 d, no longer retain this ability, and histological sections through the head of a newborn joey support the conclusion that the vestibular apparatus is undifferentiated<sup>5,6</sup>. It seems to us that this transient righting reflex could be mediated by muscle stretch receptors, particularly in the neck.

These experiments confirm the negative geotropism of the newborn quokka and reveal no other mechanism directing its movement towards the pouch. In the second experiment, in particular, the joey passed within 1 cm of the pouch opening without hesitating in its upward climb and it is clear that chemotaxis can be discounted in this case.

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## Impaired acquisition of a passive avoidance response after lesions induced in the locus coeruleus by 6-OH-dopamine

Crow<sup>1</sup> and Kety<sup>2</sup> have advanced the hypothesis that the fine network of noradrenaline-containing nerve terminals which innervates the cerebral cortex<sup>3</sup> is a necessary component of the mechanism of learning. In particular, it is suggested that these neurones deliver a "results of action"<sup>4</sup> signal which registers the successful outcome of a particular behavioural sequence. The noradrenaline released in the cortex is believed to interact with a transient synaptic change resulting from recent neural activity (the 'short-term trace'), to initiate the long term synaptic changes presumed to underlie learning. Various authors have suggested the existence of such a "confirming reaction"<sup>5</sup>, "results of action"<sup>6</sup>, or "now print"<sup>7</sup> mechanism. The diffuse distribution of noradrenergic terminals in the cerebral cortex would allow this system to deliver a generalised message of the type required. Ungerstedt<sup>7</sup> has demonstrated histochemically that the cortical noradrenergic terminals originate from cell bodies of the nucleus locus coeruleus in the dorsal pontine tegmentum, and this has been confirmed in biochemical studies<sup>8,9</sup>. The concept of the coeruleo-cortical noradrenergic system as a reinforcement pathway is supported by the observation that rats with electrodes in the region of the locus coeruleus can be trained to self stimulate<sup>10,11</sup> and this behaviour is accompanied by increased noradrenaline turnover in the ipsilateral cortex<sup>12</sup>.

The prediction that bilateral lesions of the locus coeruleus would impair learning capacity<sup>1,2,13</sup> has been tested in a runway<sup>8</sup> and a two-choice discrimination learning test<sup>14</sup>.

Since moderately extensive lesions of the locus coeruleus did not impair acquisition in this latter task it has been argued<sup>14</sup> that the apparent impairment in runway performance either does not constitute a learning deficit, or is not representative of a general impairment of learning capacity. By contrast, in an avoidance situation, Stein *et al.*<sup>15</sup> have shown that the learning deficit which can be demonstrated following administration of diethyldithiocarbamate (DDC), a drug which depletes noradrenaline stores by inhibiting dopamine- $\beta$ -hydroxylase, can be reversed by intraventricular administration of noradrenaline. These findings suggest that the integrity of certain central noradrenergic pathways is necessary for learning in this situation. Here we have investigated whether this deficit in avoidance learning is a consequence of impaired transmission in the coeruleo-cortical noradrenergic system.

Seventy-nine female Sprague-Dawley rats (initial weight 150 g) were divided into an untreated control group ( $n=24$ ), a group in which the coeruleo-cortical system was damaged by a stereotaxic injection of 6-OH-dopamine (6-OH-DA) (16  $\mu$ g in 4  $\mu$ l, bilaterally over 4 min) delivered through burr holes just anterior to the locus coeruleus 3 weeks before testing ( $n=23$ ); a group with burr holes but no injection ( $n=23$ ); and a group treated with DDC 300 mg  $\text{kg}^{-1}$  between the first and the second trials of the training procedure ( $n=9$ ). The time taken for each animal to step off a platform on to the electrifiable grid floor of the test chamber was recorded by contact switches in each of five test sessions. The first two trials were separated by 2 h, and after 1 h all animals were given either DDC or saline subcutaneously (Fig. 1). On the third trial, animals were shocked (4 mA, scrambled across the grid, for 3 s) as they stepped down, and on the fourth (1 min later) and fifth (3 d later) trials the effects of this experience on the animals' subsequent step-down time was tested. With this experimental design it is likely that dopamine- $\beta$ -hydroxylase inhibition in the DDC-treated group is present at the time

Fig. 1 The experimental design DDC 300 mg  $\text{kg}^{-1}$  by subcutaneous injection.

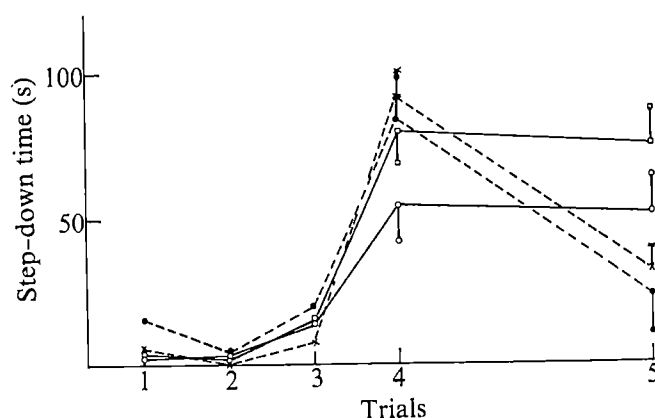
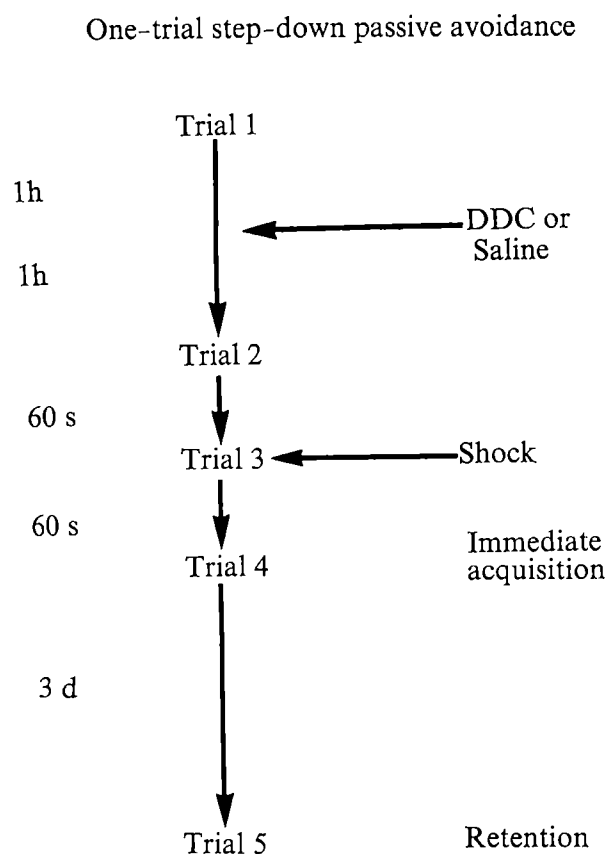


Fig. 2 Mean step-down times (s) on trials 1-5 in groups of rats treated with 6-OH-DA 3 weeks before testing (x), and DDC (300 mg  $\text{kg}^{-1}$ ) between trials 1 and 2 (●), compared with untreated controls (□) and a group of rats who had burr holes drilled in the skull (○). All animals received foot shock on stepping down on to an electrifiable grid floor on trial 3. The change in step-down time between trials 3 and 4 ( $t_4-t_3$  in Table 1) represents the effects of shock on the animal's behaviour after 60 s (immediate acquisition). The comparison between trials 4 and 5 ( $t_4-t_5$ ) is an index of the animal's ability to retain the avoidance response after 3 d (retention). Bars indicate s.e.m.

of the learning experience (trial 3) and at the time of the immediate acquisition test (trial 4), but is not present on trial 5 (ref. 15).

Both the 6-OH-DA- and DDC-treated rats showed normal immediate acquisition of the avoidance response as assessed by an increase in step-down time between trials 3 and 4 (Fig. 2 and  $t_4-t_3$  in Table 1). Neither group differed significantly from the untreated controls, although the 6-OH-DA-treated group somewhat surprisingly performed significantly better than the burr-hole group (Table 1). Compared with both the untreated controls and the burr-hole group, the two experimental groups showed a failure of retention after 3 d, as shown by the differences in step-down time between trials 4 and 5 ( $t_4-t_5$  in Table 1). Compared with the two control groups, the 6-OH-DA-lesioned and DDC-treated rats both showed a significant ( $P<0.01$ ) decrease ( $t_4-t_5$  in Table 1) in time spent on the platform. Activity levels measured in an Animex instrument (LKB Farad) showed no significant differences between the 6-OH-DA-lesioned rats, the group with burr holes and the controls (Table 2). Subsequently, the brains of the rats in the 6-OH-DA-lesioned group were removed and the cortices dissected out and assayed for noradrenaline using a spectrofluorimetric technique. The mean cortical noradrenaline level in the 6-OH-DA-lesioned group ( $n=23$ ) was  $93 \pm 12.6$  (s.e.m.) ng  $\text{g}^{-1}$ , approximately 15% of that present in a sample of the control group,  $611 \pm 68$  ng  $\text{g}^{-1}$  ( $n=4$ ).

Since immediate acquisition in these rats is unimpaired

Table 1 The increase ( $t_4-t_3$ ) in step-down time immediately following foot shock on trial 3 (immediate acquisition) and the change ( $t_4-t_5$ ) in step-down time after a subsequent 3-d interval (retention)

	<i>n</i>	Immediate acquisition $t_4-t_3$ (s $\pm$ 1 s.e.m.)	Retention $t_4-t_5$
Untreated controls	24	63.7 $\pm$ 9.5	5.2 $\pm$ 10.2
Burr holes only	23	43.6 $\pm$ 9.7	-0.9 $\pm$ 16.6
6-OH-DA lesions	23	83.2 $\pm$ 9.0†	58.5 $\pm$ 10.4*
DDC 300 mg $\text{kg}^{-1}$	9	63.6 $\pm$ 12.2	60.8 $\pm$ 17.0*

\*  $P < 0.01$  compared with untreated controls and burr-hole group  
†  $P < 0.01$  compared with burr-hole group.

**Table 2** Small and large movements measured in an Animex instrument over a period of 5 min

	<i>n</i>	Controls 24	Burr holes 23	6-OH-DA lesioned 23
Movements	Large	54±4	64±6	55±6
	Small	154±6	162±10	166±9

it seems unlikely that the deficit is due to impaired sensory or motor capacity; more probably it represents a defect in the capacity to form a long lasting record of previous experience. Moreover, since an identical impairment is observed after both administration, at the time of the learning experience, of drugs known to impair the synthesis of noradrenaline and after neurochemical lesions of a central noradrenaline-containing pathway, it seems likely, as Stein *et al.*<sup>15</sup> suggest, that the deficit results from impaired central noradrenergic transmission at a critical stage in the learning process. Our results are compatible with the hypothesis that the system of noradrenergic neurones arising from the locus coeruleus is necessary for initiating the transition from a short to a long term memory trace, and that the integrity of this system is necessary for this transition to occur, at least in an avoidance learning situation.

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## Generalisation of the Hardy-Weinberg law

THE distribution of genotypes  $q^2aa + 2qpaA + p^2AA$ , commonly called the Hardy-Weinberg law, has been recognised at least since 1908. It gives the frequencies of genotypes in a randomly mating population with respect to a single locus maintaining two alleles  $a$  and  $A$  with respective frequencies  $q$  and  $p$ . A generalisation given in 1922 by Wright and discussed in his book<sup>1</sup> gives the distribution

$$(q^2 + Fqp)aa + 2(1-F)qpaA + (p^2 + Fqp)AA$$

where  $F$ , the "fixation index" is the correlation between pairs of gametes uniting to form zygotes.

Here we point out that a distribution of the same form as Wright's is maintained in equilibrium by a pattern of assortative mating devised in 1918 by Fisher<sup>2</sup> and elaborated in 1939 and 1948 by Malécot<sup>3</sup>.

Fisher was concerned with continuously varying characters

and took as his datum that the correlation between mates could be described by a bivariate normal distribution with coefficient  $\mu$ . He found that if females with standardised value  $x$  occur in the population with frequency  $N$  and males with value  $y$  have frequency  $M$ , then  $x$ - $y$  pairs occur with frequency  $MN(1 + \mu xy)$ . In populations with random mating the corresponding frequency is  $MN$ , so that, if  $\mu > 0$ , Fisher's assumption implies a higher frequency of pairs when both partners have above average or below average values, and lower when one has above and the other below average values, compared with the randomly mating population.

Malécot<sup>4</sup> started from this implication and derived the distribution for one locus,  $f_0 = (q^2 + \lambda qp)$ ,  $f_1 = 2(1 - \lambda)qp$ ,  $f_2 = (p^2 + \lambda qp)$ , which is of the same form as Wright's, the only difference being the replacement of Wright's fixation index by Malécot's "constant of homogamy"  $\lambda$ . If the genotypes are given arbitrary values 0, 1, 2, respectively, the mean and variance of Malécot's distribution are  $m = 2p$  and  $s^2 = 2(1 + \lambda)qp$ , respectively. Also, the deviations from the mean of the genotypes are  $d_0 = -2p$ ,  $d_1 = q - p$ , and  $d_2 = 2q$ , respectively.

Consider a population which starts with Malécot's distribution in both sexes and assume that the frequencies of  $x$ - $y$  mating pairs are given by

$$f_{xy} = f_x f_y (1 + \mu d_x d_y / s^2)$$

where  $x = 0, 1, 2$  and  $y = 0, 1, 2$ . Then it can be shown that, given the usual Mendelian assumptions, the distribution of genotypes among offspring is again that of Malécot, provided that  $\mu$  and  $\lambda$  are related by  $\mu = 2\lambda/(1 + \lambda)$ . Furthermore, it is found that, for all mating frequencies to be non-negative,  $\mu$  must be restricted to the range  $(1 - 1/p^2, 1)$  if  $q \leq p$ , and  $(1 - 1/q^2, 1)$ , if  $q > p$ .

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## Cross-modal transfer in the chimpanzee

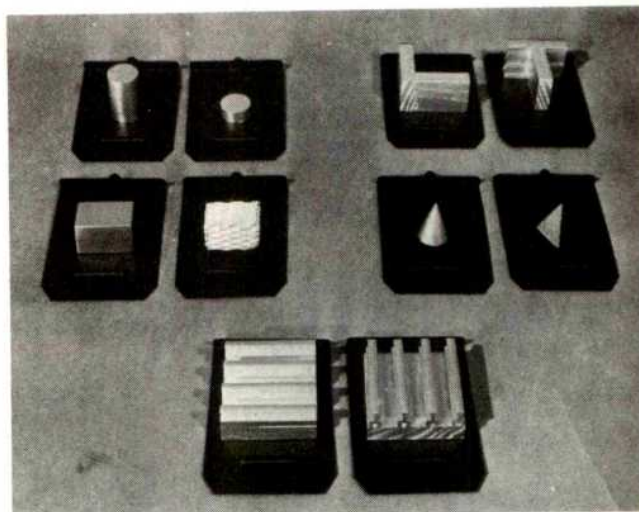
TASKS of cross-modal performance assess the capacity to recognise stimulus-objects with one of the senses when they have previously been experienced only with another. In a number of recent publications<sup>1-3</sup>, it has been shown that apes succeed at such tasks, in marked contrast to the negative or weak effects previously found for monkeys. The training procedures used with the apes were, however, new<sup>1</sup>, and had not been tried with monkeys, so that it remained possible that monkeys would perform as well as apes with these procedures<sup>4-5</sup>. Monkeys have been shown capable of cross-modal performance in two different methods of training (ref. 6, and M. J. Jarvis and G. Ettlinger, unpublished) one of which was designed to follow the principles used successfully with apes. We here describe the performance of three chimpanzees on cross-modal tasks of a kind which have in the past not yielded positive results with monkeys: our findings with the chimpanzees are unclear, or at least not certainly positive.

We assessed cross-modal transfer in three male chimpanzees (*Pan troglodytes verus*) weighing 16-20 kg, using procedures which have been described in earlier reports on monkeys<sup>7-9</sup>. The sequence of training is shown in Table 1. After preliminary



adaptation to making two-choice simultaneous discriminations by pushing lids of food boxes in a WGTA in the dark and light, the apes were trained on five transfer tasks. Each task was presented to one sense until the animal reached a standard level of performance (45 correct responses in 50 successive trials for the initial four tasks, 160 correct responses in 200 trials for task 5); and then the same pair of objects was presented to the other sense, either with the same object rewarded as before (non-reversal) or with the previously unrewarded object now rewarded (reversal). In tactile training the objects were palpated directly in total darkness. Performance was monitored with a video system sensitive to infrared light. For visual training the objects were mounted on similar lids of food boxes, but a small handle was put on the lid in front of the object. These handles had to be used in the preliminary colour discrimination (as the colours were flat papers) and subsequently the handles were used (instead of the objects) for pushing the lids on all visual trials except three. The test objects are shown in Fig. 1; 40–60 trials were given daily, five days each week, with two peanuts as reward for a correct response. The poor learning scores may reflect concurrent daily training in three other investigations: after completing its training for this experiment early each morning each ape was later trained on the same day on memory, sorting and cross-modal recognition tasks. (Observations did not suggest that  $\approx 200$  trials were too many for each animal on each day. We prefer, with Warren<sup>10</sup>, to suppose that an ape does not learn more readily than a monkey—and may learn more slowly.)

The learning scores are shown in Table 1. If the animal failed to relearn within 300 trials in the second sense, training was discontinued. Cross-modal transfer could become manifest in either (1) higher learning scores in the second sense on tasks involving reversal of reward than on non-reversal tasks; (2) lower savings scores for the second sense at reversal rather than non-reversal tasks, and/or (3) a greater number of errors in the initial ten trials in the second sense for reversal than non-reversal tasks. The mean learning scores in the second modality



**Fig. 1** Stimulus objects used for the visual versions of five transfer tasks. The objects were painted silver and set back on the lids of the food container behind small handles. The handles were used by the apes to push the lids so that no tactile information was gained during visual training in the light. For the tactile versions identical objects were painted matt black and fixed closer to the animal on another set of lids. The animals touched the objects directly to push the lids in the dark, and never gained visual experience of the objects. The tasks are identified in Table 1.

are 115 trials for 8 reversal and 120 trials for 6 non-reversal tasks. The mean savings are calculated from

$$\frac{1^{\text{st}} \text{ hand} - 2^{\text{nd}} \text{ hand}}{1^{\text{st}} \text{ hand} + 2^{\text{nd}} \text{ hand}}$$

to be  $-18.5$  for 8 reversal and  $+7.2$  for 6 non-reversal tasks. The mean errors in the first ten trials of the second modality are 6.4 errors for 8 reversal and 4.5 for 6 non-reversal tasks. After training on the transfer task 5 the procedure of Moffett and Ettlinger<sup>8</sup> was adopted for Algie and Blue: the apes were given 80 trials at random in the light and dark with the opposite orientations rewarded exactly as during learning in each modality (that is, choice of sagittal rewarded in the dark and of coronal in the light for Algie). During these interleaved visual and tactile trials the animals made 11 and 15 errors, representing the same level of performance as achieved during learning within one modality.

The findings with these three chimpanzees provide no clear evidence of cross-modal transfer. Our other chimpanzees are to be tested exactly as those described in this report but only after they have received prolonged training for cross-modal recognition according to the procedure of Jarvis and Ettlinger (unpublished). The expectation is that they will then give evidence of good cross-modal transfer. Such a finding would, in agreement with other work<sup>1</sup>, indicate that the chimpanzee can indeed attain cross-modal performance as a consequence of lengthy training. This is, however, also true for the monkey (Jarvis and Ettlinger, unpublished). Moreover, with the method of Cowey and Weiskrantz<sup>9</sup> monkeys and chimpanzees<sup>11</sup> can additionally attain unidirectional cross-modal recognition without lengthy training. We therefore conclude that it remains to be shown whether important differences exist between the cross-modal capacities of apes and monkeys. The present experiment emphasises the importance of testing different species in truly comparable conditions.

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**Table 1** Training sequence and performance for 3 chimpanzees on 5 cross-modal tasks

		Bertie	Algie	Blue
Adaptation				
Cylinder/Sphere	Tactile	720	420	120
Blue/Orange	Visual	190	290	300
First transfer test				
small/tall	Tactile	90	140	30
(size)	Reversal	270*(7)	90 (3)	Reversal
	Visual			60 (7)
Second transfer test				
smooth/rough	Visual	0	0	90
(roughness)	Tactile	20 (7)	Reversal	0 (1)
			190†(4)	
Third transfer test				
L/T	Tactile	690	510	190
(size)	Visual	230 (5)	Reversal	80 (4)
			120 (8)	
Fourth transfer test				
pyramid/cone	Visual	380	50	120
(roughness)	Reversal	40 (6)	220*(7)	Reversal
	Tactile			60 (9)
Fifth transfer test				
sagittal/coronal	Tactile	600 failed	0	0
(orientation)	Visual	—	Reversal	Reversal
			0 (4)	40 (6)
Tactile and visual				
at random				
Errors in 80 trials		—	(11)	(15)

\* Tests up to 27/30, within 300 trials.

† Tests to 35/40, within 300 trials.

Numbers give learning scores (up to 45/50, except on the fifth test when it was 160/200). Numbers in parentheses give errors on the first 10 trials in the new sense, except for lowest row where they represent errors in 80 trials of interleaved tactile and visual training with reward for reversed response. Scores for the second sense refer to non-reversed response, unless indicated.

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## Novel surface polymer changes in development of *Myxococcus* spp.

THE Myxobacteriaceae are unique among prokaryotes in their development cycle. Vegetative cells are Gram-negative bacilli which undergo binary fission as in other prokaryotes; alternatively, the cells can aggregate on solid surfaces to form fruiting bodies in which the bacteria undergo conversion to myxospores. In *Myxococcus xanthus* and related species a simple fruiting body is formed in which spherical myxospores are surrounded by extracellular polysaccharide slime<sup>1</sup>. In at least one strain of *M. xanthus*, synchronous myxospore formation can be obtained in liquid culture through induction with glycerol and other agents<sup>2</sup>. The entire bacillus undergoes conversion to the myxospore form; there is no apparent loss of cell material such as is observed in the morphogenesis of Gram-positive bacilli to endospores at the expense of cell wall and surface material shed on emergence of the mature spore. The fate of the *Myxococcus* surface polymers is thus of particular interest.

*M. xanthus* has been the subject of numerous cytological studies<sup>3,4</sup> which have indicated the formation of a thick extracellular coat together with a vesicular structure contained between this coat and the cytoplasmic membrane. The structural polymer, peptidoglycan, is present in cell walls of both bacilli and myxospores, although in a slightly modified form in the latter<sup>5</sup>. Both bacilli and myxospores are surrounded on solid media by extracellular polysaccharide which is of the same chemotype, and possibly identical, in bacillary cultures and in fruiting bodies<sup>6</sup>. We have now investigated the cell wall polysaccharides in bacilli and in myxospores, both those isolated from fruiting bodies and those obtained by glycerol induction. In particular, myxospores have been examined to determine whether they contained lipopolysaccharide.

Bacteria used were *M. xanthus* strain FB (Professor M. Dworkin, University of Minnesota) and a strain isolated from local soil (designated S). Strain FB underwent dispersed growth in liquid media and was inducible to myxospores by glycerol; strain S gave granular growth and was only poorly inducible to myxospores. The microanalytical procedures used have been described previously<sup>6,7</sup>, except that acid hydrolysates were neutralised with Ba(OH)<sub>2</sub>, separated into neutral and hexosamine fractions by preparative paper electrophoresis in pyridinium acetate buffer (pH 5.3) and analysed after elution. This procedure was

adopted because of the difficulty in obtaining uniform suspensions of myxospores and the use of hydrolysates of whole cells.

Lipopolysaccharide was present in the bacillary forms of the two strains and was extractable by the hot phenol procedure (65 °C, 5 min). It could then be deposited by ultracentrifugation at 100,000g for 4 h. Attempts to obtain lipopolysaccharide from myxospores of either strain were unsuccessful, although the bacilli had yielded 0.7–1.1% of their dry weight as sedimentable lipopolysaccharide. No carbohydrate-containing material was present in the phenol phase after extraction of myxospores as would be expected if the polymer had become more hydrophobic through loss of polysaccharide side chains<sup>8</sup>. As myxospores cannot be disintegrated by ultrasonic treatment or other readily available methods<sup>9</sup> an alternative extraction procedure was required to obtain the polysaccharides known to be present<sup>10</sup>. The composition of these polysaccharides could be ascertained by analysis of hydrolysates of whole myxospores, as the only other monosaccharides known to be present are *N*-acetylglucosamine and *N*-acetylmuramic acid derived from peptidoglycan. The extraction procedure used by Freeman<sup>11</sup> to obtain side chain material from *Salmonella* lipopolysaccharides, 0.1 M acetic acid at 100 °C for 12 h, yielded carbohydrate-containing material from both bacilli and myxospores. From 95 mg freeze-dried myxospores of strain FB, 9 mg non-diffusible material was solubilised by the acetic acid treatment; the carbohydrate content was approximately 65%. Yields from strain S myxospores isolated from fruiting bodies were slightly lower. The carbohydrate composition of the lipopolysaccharides, acetic acid extracts and whole myxospores was compared by analysis and paper chromatography of hydrolysates (Table 1). Slight differences between the lipopolysaccharide composition found for strain FB and the results reported<sup>7</sup> are almost certainly attributable to the improved accuracy of the analytical procedures when hydrolysates were freed from salts and other non-carbohydrate material by electrophoresis.

Chromatograms indicated that in strain FB, mannose present in the lipopolysaccharide had almost all disappeared from myxospores, while the ratio of glucose and galactosamine to glucosamine increased greatly in the myxospores. No appreciable differences were found between myxospores of this strain induced with glycerol and those obtained from fruiting bodies. Strain S lipopolysaccharide contained two sugars, mannose and galactose, which were almost entirely lost from myxospores; again there was an apparent increase in the glucose and galactosamine content. These observations were confirmed by analysis.

The two *Myxococcus* strains examined lack any of the more unusual sugars reported in the lipopolysaccharides of other Gram-negative bacteria—heptose, 3-*O*-methyl xylose and dideoxyhexoses are absent. Both, however, contain 2-keto-3-deoxyoctonic acid in their lipopolysaccharides<sup>7</sup>. Attempts to identify this compound in hydrolysates of

Table 1 Composition of polysaccharide preparations from *Myxococcus* strains FB and S

Strain	Preparation	Mannose	Glucose	Galactose	Glucosamine	Galactosamine	Ribose
FB	Lipopolysaccharide	15.3	31.2	0.3	18.9	29.0	5.1
	Lipid-free polysaccharide	21.3	35.0	0.3	16.1	23.7	3.5
	Freeman extract of bacteria	15.1	50.9	0.4	13.2	18.2	2.3
	Freeman extract of myxospores	0.2	67.7	0.2	5.2	24.9	0.6
	Myxospores*	0.9	63.1	0.2	6.8	26.7	0.7
	Myxospores†	0.7	69.0	0.4	5.4	23.5	0.5
S	Lipopolysaccharide	34.1	18.8	18.1	10.1	14.8	3.9
	Freeman extract of bacteria	30.6	18.9	14.9	15.4	15.4	3.5
	Myxospores†	7.3	51.2	5.8	9.9	23.4	2.4

\*Glycerol induced; †Isolated from fruiting bodies.

Monosaccharide values are expressed as % total carbohydrate recovered after electrophoresis and elution.



myxospores were unsuccessful although it was identified on electrophoretic separation of the lipopolysaccharide hydrolysates. Although not conclusive in itself, this result, together with the loss of other monosaccharides, suggests that a hitherto unrecognised feature of the formation of myxospores from the bacillary forms of *M. xanthus* (and a phenomenon not previously reported for Gram-negative bacteria) is the complete loss from the wall of the polysaccharide portion of the lipopolysaccharide. The fate of the lipid A portion has not yet been ascertained. An analogy can thus be found to the loss of teichoic acid in bacterial endospore formation<sup>12</sup>, except that the bacillary wall is not lost during myxospore development. Instead, there is an apparently selective rejection of the polysaccharide component of the lipopolysaccharide. It is not clear whether this is attributable to enzymatic removal or to ejection to make way for the new glucose- and galactosamine-containing polymer identified by Johnson and White<sup>10</sup>. Preliminary results suggest that replacement of lipopolysaccharide by a new polymer also occurs during myxospore formation in other fruiting species of Myxobacteria.

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## Use of ionophore A23187 to measure cytoplasmic Ca buffering and activation of the Ca pump by internal Ca

DURING the formation of the mature mammalian erythrocyte, the reticulocyte sheds its nucleus, endoplasmic reticulum and mitochondria leaving a featureless cytoplasm with a high haemoglobin content surrounded by a plasma membrane. All Ca-accumulating organelles are lost and the mature red cell is maintained virtually Ca-free throughout its life<sup>1</sup> by the low Ca permeability of its membrane<sup>2</sup> perhaps aided by a powerful Ca-extrusion pump<sup>3</sup>. Most of our knowledge of Ca transport in red cells comes from studies with resealed ghosts, but in this preparation the results are often affected by alterations to the membrane and metabolism of the cell<sup>2-4</sup>. The main difficulty of using intact fresh cells in physiological conditions has been the impossibility of increasing their Ca content in a controlled way and of assessing the fraction of Ca which is ionised. Using a divalent-cation ionophore, A23187 (ref. 5), however, we have overcome these difficulties and found that cytoplasmic Ca buffering occurs as if the cell had a single large-capacity, low-affinity Ca buffer and that there are two Ca-translocating sites of equal affinity at the internal surface of the Ca pump.

Preliminary experiments showed that in the red cell the ionophore selectively increases the Ca and Mg fluxes without affecting directly the Na and K fluxes, in agreement with its action in other systems<sup>6</sup>. The incorporation of the ionophore into the red cell membrane induces a large Ca leak. In these conditions, the rate of change of the total Ca concentration in the cell ( $\mu\text{mol per l cells per h}$ ) is given by

$$d\text{Ca}_i^T/dt = P(r^2\text{Ca}_o - \alpha\text{Ca}_i^T) - \phi \quad (1)$$

where  $P$  represents the ionophore-induced leak permeability

for Ca ( $\text{h}^{-1}$ );  $r^2$  is the Donnan distribution ratio for divalent ions at equilibrium and is related to the membrane potential ( $V_m$ ) by the expression  $r^2 = \exp(-2FV_m/RT)$ ;  $\alpha$  is the fraction of ionised Ca inside the cell;  $\text{Ca}_o$  is the concentration of ionised Ca in the medium and  $\phi$  is the Ca extrusion rate through the Ca pump ( $\mu\text{mol per l cells per h}$ ). The maintenance of steady states for  $\text{Ca}_i^T$  (see Fig. 1) suggests that the Ca pump remains saturated with ATP for the duration of our experiments. Following Schatzmann<sup>3</sup> the pump-flux term can therefore be expressed as

$$\phi = \phi_m[\alpha\text{Ca}_i^T/(\alpha\text{Ca}_i^T + K)]$$

where  $\phi_m$  represents the maximum Ca pumping rate and  $K$  is the Ca dissociation constant at the internal surface of the pump.

Red cells from fresh blood or from 3-4-d-old bank blood were washed 3 times with about ten volumes of ice-cold solution containing NaCl, 75 mM; KCl, 75 mM; Tris-Cl (pH 7.5 at 37 °C), 10 mM and Tris-EGTA (1,2-Di(2-aminoethoxy) ethane-NNN'-tetraacetic acid), 0.1 mM and 4 times more with the same solution without Tris-EGTA. After the washes the cells were packed at 2,000g for 5 min and the supernatant disposed of. At  $t = 0$ , 0.25 ml of packed cells were added to 2 ml of a medium at 37 °C stirred magnetically and containing: NaCl, 75 mM; KCl, 75 mM; Tris-HCl (pH 7.5 at 37 °C), 10 mM;  $\text{MgCl}_2$ , 1-2.33 mM; Inosine, 10 mM, 5  $\mu\text{Ci}$  of  $^{45}\text{Ca}$  and varying amounts of  $\text{CaCl}_2$ . This particular K concentration was used to minimise the effect which a Ca-induced increase in K permeability<sup>6</sup> might have had on the membrane potential and, therefore, on  $r$ . Samples were withdrawn from this cell suspension at suitable time intervals and processed as detailed in the legend of Fig. 1.

Figure 1 shows two representative time curves at initial concentrations of  $\text{Ca}_o$  of 93.2 and 1,000  $\mu\text{M}$ . In this experiment, at 93.2  $\mu\text{M}$   $\text{Ca}_o$  and the lower concentrations, the uptake of Ca at 30 s went through a maximum considerably larger than the steady-state value, whereas at higher  $\text{Ca}_o$  the kinetic pattern followed was that represented by the 1,000- $\mu\text{M}$  curve. The transient peak was never observed in ATP-depleted cells<sup>7</sup> or at higher external Ca concentrations in fed cells. Its dependence on the metabolic state of the cell suggests an initial inactivity of the Ca pump. Successive additions of ionophore to fed cells in the presence of lower Ca concentrations, however, always elicits a transient peak response. A possible explanation of these peaks is that initially only a fraction of the cells takes up all the ionophore, thus behaving like the high-ionophore cells in Fig. 2. As the ionophore becomes more homogeneously distributed, Ca is pumped out from these cells and a final steady state with a lower average Ca content is achieved. If  $^{45}\text{Ca}$  is added during steady state to cells containing enough Ca to saturate the pump, the final  $^{45}\text{Ca}$  distribution is the same as in controls with  $^{45}\text{Ca}$  present from the beginning but is approached exponentially without any maxima. In depleted cells, the steady-state distribution of Ca was unaffected by further additions of ionophore, but in fed cells this was true only for very high initial ionophore concentrations (above 100  $\mu\text{mol per l cells}$ ).

The use of  $\alpha$  in equation (1) to describe the buffering of Ca by the red cell involves a number of simplifying assumptions which require consideration. In the general case for  $n$  intracellular buffers the relation between total and free Ca is described by

$$\text{Ca}_i^T = \text{Ca}_i^{2+}[(V_w/V_c) + \sum_{i=1}^n \{B_i/(K_i + \text{Ca}_i^{2+})\}] \quad (2)$$

where  $V_w$  is the volume of cell water per litre of cells ( $V_c$ );  $B_i$ , the total concentration of the  $i$ th buffer, and  $K_i$  the dissociation constant between Ca and this buffer.

In ATP-depleted cells<sup>7</sup>, the ionophore induced the red cell

membrane to act like a "dialysis bag" for Ca. The equilibrium distribution of Ca as a function of external Ca showed that Ca buffering can be satisfactorily described assuming the cells have a single large-capacity low-affinity Ca buffer in agreement with earlier results obtained with cell lysates<sup>3</sup>. In this case, equation (2) becomes

$$Ca_i^T = Ca_i^{2+}[(V_w/V_c) + B/(K_B + Ca_i^{2+})]$$

If  $Ca_i^{2+} \ll K_B$  then

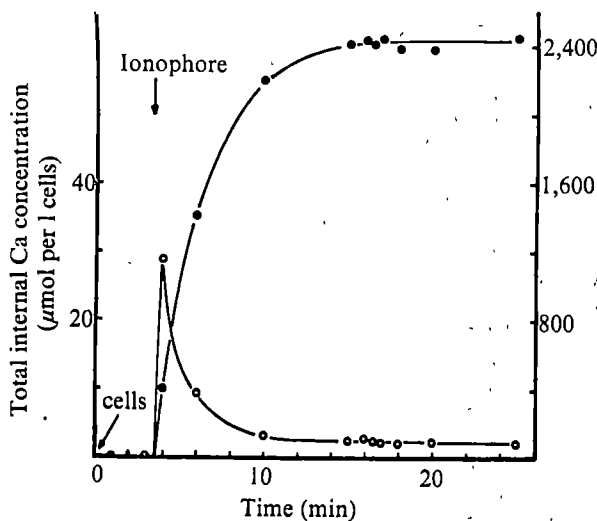
$$Ca_i^T = Ca_i^{2+}[(V_w/V_c) + (B/K_B)]$$

and

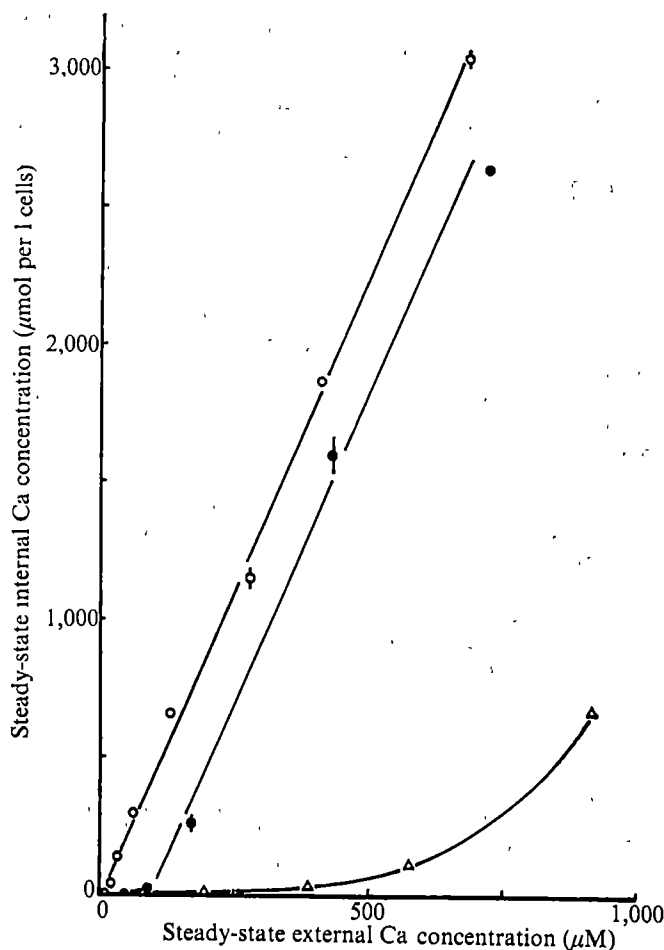
$$\alpha = [(V_w/V_c) + (B/K_B)]^{-1}$$

The use of  $V_w/V_c$  already allows for the fact that while  $Ca_i^T$  is expressed per litre of cells,  $Ca_i^{2+}$  represents the actual concentration of ionised Ca per litre of cell water.

In fresh cells, the buffering capacity was estimated from the slope of the curve relating the steady-state internal Ca concentration ( $Ca_i^T$ ) with the external free Ca concentration ( $Ca_o$ )



**Fig. 1** Ca content of cells as a function of time at two different external Ca concentrations. At the times indicated 0.1-ml samples of the cell suspension were placed in 1.5-ml Eppendorf centrifuge tubes containing 0.4 ml *n*-butyl-phthalate (BDH, density 1.042–1.045) and 0.9 ml of the initial washing medium but with 5 mM Tris-EGTA, all at 0–1 °C. Each sample was centrifuged immediately for 10 s at 12,000g in an Eppendorf centrifuge model 3200. The cells formed a compact pellet at the bottom of the oil layer and the aqueous supernatant remained on top. The whole procedure from sampling to cell separation took about 20 s. The supernatant, including the oil, was sucked off and the walls of the tube cleaned and dried with cotton swabs. The cells were lysed in 0.5 ml of distilled water, the proteins precipitated with 5% TCA and aliquots used for counting. Samples for total activity and haemoglobin were taken at the end of each run. The extracellular activity trapped in the cellular pellet was estimated using <sup>45</sup>Ca in the presence of a large excess of Tris-EGTA. The cells were added at  $t = 0$  and the ionophore was added after the second sample, at  $t = 3.5$  min in 10 μl of absolute ethanol to give a final concentration of 10 μmol per l cells (assuming complete partitioning of the ionophore into the cells). Ordinates: Ca content of cells (in μmol per l original cell volume). Note the fortyfold difference in scale between left and right ordinates corresponding to the curves at low and high external Ca concentration, respectively. ○, Initial external Ca concentration, 93.2 μM, final steady state concentration, 90.7 μM; ●, initial external Ca concentration: 1,000 μM, final steady state concentration, 740 μM. Haematocrit, 8.9%. The increased frequency of sampling during the steady state is due to the fact that <sup>42</sup>K was added at  $t = 15.5$  min to measure simultaneously the K influx associated with each particular steady Ca level. (The results on the relationship between potassium permeability and the internal free Ca concentration in the intact fresh red cell will be published elsewhere.) The curves represent the kinetic patterns obtained with eleven concentrations of external Ca tested (see text).



**Fig. 2** Total Ca content of red cells as a function of external Ca concentration during steady state. In this experiment we used three ionophore concentrations: ○, 100 μmol per l cells,  $I = 100$ ; ●, 25 μmol per l cells,  $I = 25$ ; and △, 6.3 μmol per l cells,  $I = 6.3$ . Assuming complete partitioning of the ionophore into the cells, the highest concentration corresponds to about  $6 \times 10^8$  molecules per cell. If it is all partitioned into the red cell membrane, at the highest concentration there would be roughly one molecule of ionophore per 2,600 Å<sup>2</sup> of membrane which, with a molecular weight of 523 (ref. 5), will cover probably less than 0.5% of the cell surface. The value of  $\alpha$  in this experiment was 0.43 and it was unaffected by the concentration of ionophore used. It was assumed that  $r^2 = 2$  which corresponds to a steady-state membrane potential of about 10 mV.

when the Ca pump became saturated (see Fig. 2). When  $dCa_i^T/dt = 0$  (steady state) and  $\phi = \phi_m$  equation (1) becomes

$$Ca_i^T = (r^2/\alpha)Ca_o - (\phi_m/\alpha P) \quad (3)$$

This relation between  $Ca_i^T$  and  $Ca_o$  was shown to be valid over a wide range of ionophore and Ca concentrations (see Fig. 2). The value of  $\alpha$  varied from 0.3 to 0.5 among cells from different donors indicating that the fraction of ionised Ca represents between 30% and 50% of the total intracellular Ca concentration.

We then calculated the value of the dissociation constant for Ca at the internal pump site. At relatively low external Ca concentrations, the Ca pump is capable of maintaining large Ca gradients across the membrane during steady state (see Fig. 1), and in these conditions  $dCa_i^T/dt = 0$  and  $r^2Ca_o \gg \alpha Ca_i^T$ . Equation (1) can be rearranged to give

$$Ca_o = (\phi_m/r^2P)[\alpha Ca_i^T/(K + \alpha Ca_i^T)] \quad (4)$$

At similar external concentrations, equation (4) can be used to compare the measured value of  $\alpha Ca_i^T$  from different experiments and at different ionophore concentrations. If  $x$  and  $y$  represent the ionised Ca concentration ( $\alpha Ca_i^T$ ) obtained in two different

experiments, or at two different ionophore concentrations, then, equation (4) becomes

$$Ca_0 = C_1[x/(K+x)] = C_2[y/(K+y)] \quad (5)$$

where  $C_1$  and  $C_2$  represent the constant factor ( $\phi_m/r^2P$ ) for the two conditions that are being compared. Equation (5) can be rearranged to give

$$1/x = (C_1/C_2)(1/y) + [(C_1/C_2) - 1]/K \quad (6)$$

This equation predicts a linear relationship from which the value of  $K$  can be calculated. Figure 3 illustrates such a calculation and confirms the linear relation predicted by equation (6). The values of  $K$  obtained by this method varied between 0.73 and 1.02  $\mu\text{M}$ . Note that  $K$  calculated in this way is independent of the stoichiometry of transport, since it remains invariant if equation (5) were

$$C_1[x/(K+x)]^n = C_2[y/(K+y)]^n$$

provided that the different translocating sites have similar affinities.

This value of  $K$  was then used to plot  $(r^2Ca_0 - \alpha Ca_i^T)$  as a function of the pump saturation ratio  $[\alpha Ca_i^T/(K + \alpha Ca_i^T)]$  in all the conditions tested (Fig. 4). The linear relationship predicted by equation (1) was only confirmed when we used a stoichiometry of two translocating sites (see Fig. 4).

The slope of the curve in Fig. 4 (slope =  $\phi_m/P$ , equation (1)) could be used to estimate  $\phi_m$  if  $P$  were known. We can only obtain a rough estimate of  $P$  from the ratio between the  $\text{Ca}$  uptake at 30 s and  $r^2Ca_0$ . This gives values of  $P$  between 7 and 20  $\text{h}^{-1}$  which correspond to values of  $\phi_m$  of about 7 to 12  $\text{mmol per l cells per h}$ . This is considerably lower than the value expected at 37 °C from Schatzmann's data in resealed ghosts<sup>3</sup>.

This study introduces a general method by which the  $\text{Ca}$  concentration of the intact red cell can be varied in a controlled manner and its ionised fraction assessed. The method could also be applied to studies with  $\text{Mg}$  and opens new possibilities for the investigation of all the mechanisms involving divalent cations in the red cell. We report here a first application of this method. The binding of  $\text{Ca}$  at the internal  $\text{Ca}$ -pump surface can be described as taking place at two sites with similar dissociation constants around 1  $\mu\text{M}$ . This value closely agrees with a half-saturation concentration of 4  $\mu\text{M}$  or less for  $\text{Ca}$  translocation reported by Schatzmann<sup>3</sup>. Schatzmann, however, suggested a stoichiometry of one  $\text{Ca}$  per  $\text{ATP}$  hydrolysed and although we have not yet measured the hydrolysis of

Fig. 3 The relationship between the reciprocals of the steady-state intracellular concentrations of  $\text{Ca}$  at similar low external  $\text{Ca}$  levels of the two lower ionophore curves of Fig. 2. The continuous line corresponds to the equation

$$1/x = -0.179 + 0.577(1/y); K = 1.02$$

and is interpreted according to equation (6) in the text.

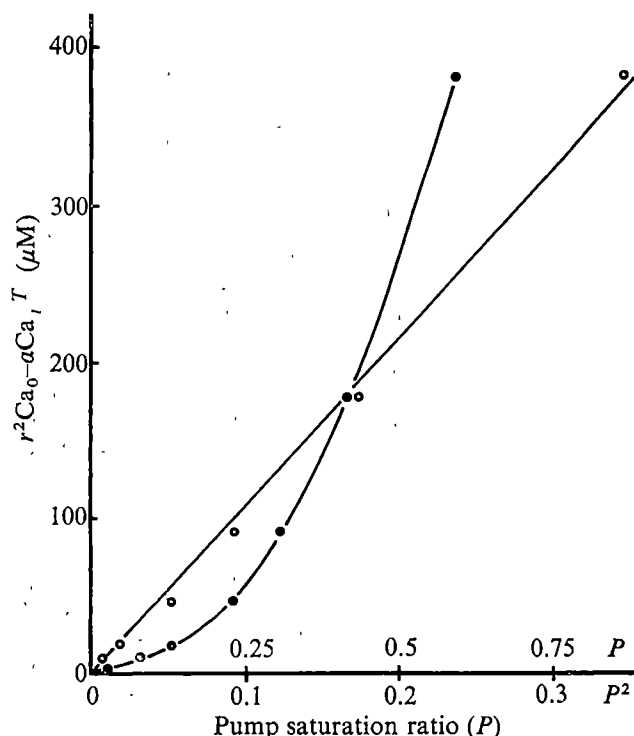
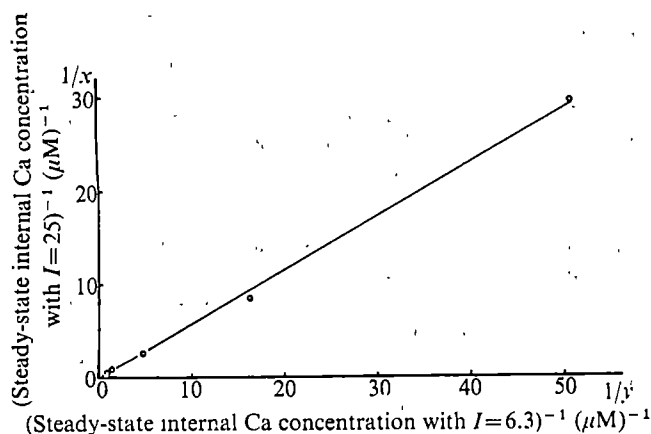


Fig. 4 The relationship between the steady-state value of  $(r^2Ca_0 - \alpha Ca_i^T)$  and the pump saturation ratio

$$P = [\alpha Ca_i^T / (K + \alpha Ca_i^T)]^n$$

according to equation (1). The value of  $K$  used was 1.02  $\mu\text{M}$  as obtained from Fig. 3. It can be seen that the straight line relationship predicted by equation (1) is obtained only if the square of the pump saturation ratio is used. This curve, corresponds to the curve obtained with the lowest ionophore concentration of Fig. 2. ●,  $n = 1$ ; ○,  $n = 2$ .

$\text{ATP}$  associated with  $\text{Ca}$  extrusion, our results firmly support a translocating stoichiometry of two  $\text{Ca}$  ions per pump cycle. This suggests a closer similarity with the sarcoplasmic reticulum pump<sup>7</sup>.

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## L-leucine, a specific inhibitor of a rare human placental alkaline phosphatase phenotype

FISHMAN *et al.* reported that human placental alkaline phosphatase (Regan isoenzyme) was found in the serum and tumour tissue of a patient with lung cancer<sup>1</sup>. Two electrophoretically different variant forms of Regan alkaline phosphatase with a greater sensitivity to inhibition by L-leucine have since been described: the 'Nagao isoenzyme'<sup>2</sup>



and a hepatoma-specific alkaline phosphatase<sup>3-5</sup>. Inglis *et al.*<sup>6</sup> reported that the L-leucine-sensitive Nagao isoenzyme found in some cancer patients closely resembled the 'D variant' of human placental alkaline phosphatase, a rare phenotype defined electrophoretically on starch gel which is found in less than 1% of the general population of pregnancies<sup>7</sup>. An example was reported of a pregnancy serum D-phenotype enzyme presumably of placental origin, confirmed by electrophoresis established as L-leucine-sensitive<sup>6</sup>. Most interesting was the occurrence of the variant at high frequency in the ovarian cancer patients surveyed<sup>6,8</sup>. To extend the observations of Inglis *et al.*, screening for the phosphatases in placenta was carried out, both to establish the frequency of the L-leucine-sensitive placental phenotypes (which should match the frequency of the electrophoretically defined D variant phenotypes) and to provide enough enzyme to enable purification and characterisation. Supply of cancer tissue is generally restrictive for this purpose.

Using a sample of ovarian cancer ascitic fluid containing the L-leucine-sensitive variant<sup>6</sup> as reference standard, we found nine placentae out of a total of 2,323 placentae screened (frequency 0.0039) which closely resembled the cancer patient enzyme in sensitivity to L-leucine. Normal placentae were obtained from St Margaret's Hospital, Dorchester, immediately after delivery and stored at -20 °C. Trophoblast tissue (5-15 g) was excised from each frozen placenta using a bone scalpel and homogenised with two volumes 0.9% saline per g tissue. The homogenate was centrifuged at 6,000g for 10 min and the supernatant diluted 1:100 with 0.01 M Tris-HCl-buffered saline, pH 8.3.

Alkaline phosphatase assay was carried out as described previously<sup>9</sup> using 18 mM phenyl phosphate as a substrate in 0.05 M sodium carbonate-bicarbonate buffer, pH 9.8, containing 10 mM MgCl<sub>2</sub>. To determine the optimum concentration of L-leucine, a partially purified enzyme preparation from an ovarian cancer patient was assayed for L-leucine inhibition. It was found to be 43% inhibited at a concentration of 0.5 mM L-leucine, using 0.5 mM D-leucine as a reference, whereas a non-variant placental enzyme was inhibited 8%. L-leucine (0.5 mM) was therefore used for screening, using the same concentration of D-leucine in the control sample. Each diluted sample (100 µl aliquots) was assayed for 30 min at 37 °C in 0.5 mM D- and L-leucine-containing substrate solutions. Inhibition of the 2,314 normal placentae was  $7.8 \pm 4.3\%$ , demonstrating that the common phenotypes of placental alkaline phosphatase<sup>7</sup> are not inhibited by L-leucine. Only the nine variants were outside the normal range (Table 1). Average inhibition profiles for the enzymes from nine individual variants and five normal placentae are shown as a function of L-leucine concentration. At all L-leucine concentrations tested, the variant enzymes were different from normal, this difference reaching a maximum of 40% at 0.5-2.0 mM L-leucine.

If the variant placentae are heterozygotes<sup>7</sup> consisting of 50% common phenotypic enzyme and 50% L-leucine-

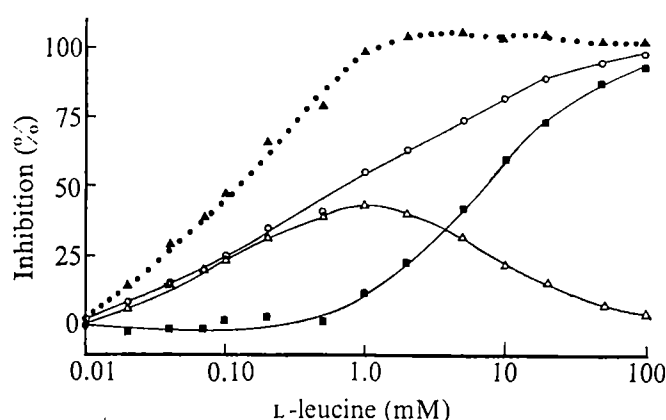


Fig. 1 Average inhibition profiles in increasing concentrations of L-leucine: ○, variant enzyme (cv); ■, normal enzyme (cc); △, difference between inhibition of variant and normal enzymes (cv-cc); ▲, difference between twice the inhibition of heterozygous variant enzyme and normal enzyme (2cv-cc).

sensitive variant enzyme, it would be reasonable to estimate the inhibition of the L-leucine-sensitive moiety alone (vv) from the expression:

$$2cv - cc = vv$$

where cv represents the inhibition of the heterozygote made up of the common and variant phenotypes, cc the inhibition of placentae containing only the common phenotypes, and vv the inhibition expected for a homozygous variant enzyme. The approximation used here makes two assumptions: that the turnover number of the variant and common phenotypic enzymes for 18 mM phenyl phosphate are identical, and that there is no cooperativity in the inhibition of normal and variant enzyme in the heterozygote.

The result of this calculation for a full range of L-leucine concentrations is shown in Fig. 1 as the dotted line. The curve for vv reaches a level of 100% at about 1 mM L-leucine (the concentration at which the difference between the common and variant enzymes is maximal), and remains constant at 100-105% between 1 and 100 mM L-leucine. By comparison with the common placental profile cc, which reaches a value of 50% inhibition at an L-leucine concentration of 7.3 mM, the vv profile shows an inhibition of 50% at about 0.12 mM, or a 60 times lower concentration of L-leucine. This difference in the concentration of L-leucine which inhibits 50% is a convenient reference value for comparison of the common cc and variant vv inhibitions. Similarly, the difference between the inhibition of the heterozygous variant and common placental enzymes at the L-leucine concentration at which this difference is maximal (that is, 0.5-2.0 mM L-leucine) can also be regarded as a basis for comparison of the variant enzymes of placentae and cancer patients. Such a comparison is made in Table 1—reference cancer fluid and nine placental enzymes found.

Since the cancer patient enzyme was inhibited to the same extent as the heterozygote placental variants in 0.5 mM L-leucine, the leucine-sensitive Nagao isoenzyme in the sera and the fluid of cancer patients can be assumed to reflect the expression of both common and variant genes, and therefore to be a heterozygote. A similar degree of L-leucine inhibition has been reported for the Nagao isoenzyme<sup>2</sup> and for the electrophoretically fast-moving hepatoma-specific alkaline phosphatase<sup>4,5</sup>.

In connection with the greater L-leucine sensitivity of the variant, note that the stereospecific inhibition of mammalian alkaline phosphatase by L-amino acids<sup>13,14</sup> is not reported for any prokaryotic alkaline phosphatases. The variant enzyme described in this work should enable a more detailed study of this mode of inhibition; much

Table 1 Inhibition of extracts of nine variant placentae by 0.5 mM L-leucine, using 0.5 mM D-leucine as control compared with cancer ascites fluid

	% Inhibition (±s.d.)
Cancer ascites fluid	46.2±0.8
Variant 1	45.8±1.6
Variant 2	45.6±1.0
Variant 3	43.5±0.7
Variant 4	46.2±0.7
Variant 5	48.6±1.6
Variant 6	47.0±1.0
Variant 7	44.5±1.3
Variant 8	48.8±1.0
Variant 9	48.7±1.2

Assays were run in pentuplicate. Cancer ascites fluid provided by Dr L. L. Stolbach contained exclusively the variant enzyme (Nagao).

as the availability of mutant forms of bacterial enzymes, which are less feedback-inhibited by amino acids, has facilitated the study of feedback inhibition<sup>15</sup>.

The relationship of the variant enzyme to cancer is implicit in the similarity of the leucine sensitivity of the cancer patient enzyme with that of the placental variant, and in the similarity in immunoreactivity of this enzyme<sup>8</sup>. These two observations alone could not, however, be regarded as proof that the two enzymes represent the same genomic origin, or that the enzyme may mask a predisposition to certain cancers. Detailed structural and kinetic studies are being carried out to determine whether the variant and ascites enzymes are in fact identical in all respects. Additionally, epidemiological studies on the occurrence of various types of cancer in the families of children bearing the variant enzyme gene will be necessary.

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## Late acquisition of a germ line antibody specificity

THE humoral immune response to most antigenic determinants is characterised by a highly heterogeneous population of antibody molecules derived from a large array of precursor cell clonotypes<sup>1-3</sup>. The response to certain antigenic determinants, however, is relatively restricted and may be dominated by a single homogeneous antibody species. In inbred strains of mice, such dominant antibody specificities are present in all individuals of a strain<sup>4-9</sup>, are characterised by identical idiotype determinants<sup>4-8</sup>, and are linked to heavy chain allotype markers<sup>7-10</sup>. Thus, these clonotypes are presumed to be a direct reflection of genetic information present in the germ line. A good example of an antibody specificity considered to be 'germ line' is the major clonotype responsive to phosphorylcholine in BALB/c mice, which is identical in its combining region to the TEPC 15 and S107 plasmacytoma proteins<sup>4-5</sup>. Representatives of the cell clone producing this phosphorylcholine-specific antibody have been found in high frequency (19±6 per 10<sup>6</sup> bone marrow-derived antibody-forming cell precursors (B cells)) in both conventionally reared and germ-free BALB/c mice<sup>11</sup>.

'Germ line' specificities can be enumerated by analysing the specificity repertoire of neonatal mice<sup>3,12,13</sup>. Since few

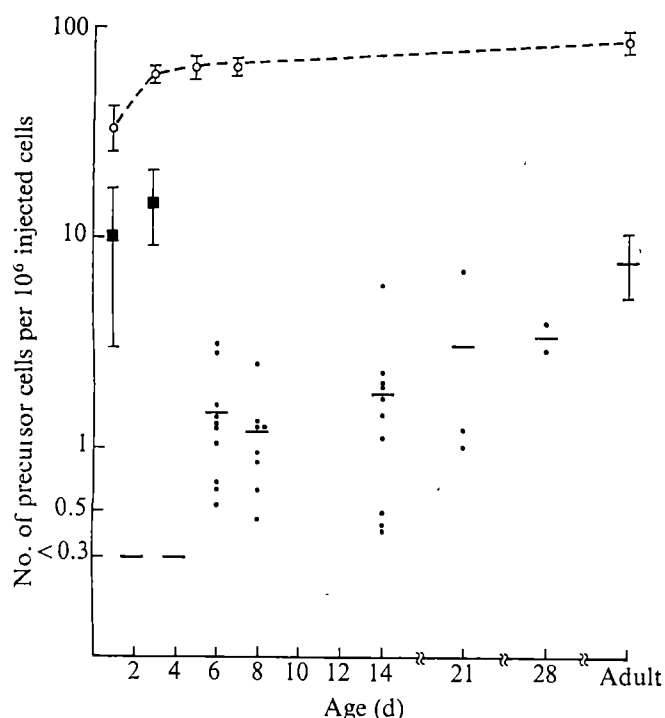
B cells are present during the first few days after birth, the neonatal repertoire may be thought to be quite restricted. Examination of the available clonotypes specific for the haptens dinitrophenol (DNP) and trinitrophenol (TNP) immediately after parturition revealed at least six clonotypes, three specific for DNP and three for TNP, present repeatedly in different individuals<sup>13</sup>. These predominant clonotypes can be represented by many (>100) cells. Other clonotypes specific for DNP or TNP occur only sporadically in the early neonatal B-cell population. By the seventh day after birth, however, such sporadic clonotypes represent most of the precursor pool, and presumably represent clonotypes expressed later in development than the early predominant ones<sup>13</sup>. It is conceivable that the all or none nature of the neonatal response to antigens like fluorescein<sup>3,13</sup> and the temporal hierarchy observed in the appearance of foetal and neonatal responses<sup>14,15</sup> may be accounted for by sporadic or late occurring clonotypes.

Since precursor cells representing the early predominant clonotypes occur with great regularity among individuals, and are present in large numbers at birth, they presumably were initiated early in foetal life and thus may be close reflections of the germ line genetic information for antibody specificity<sup>13</sup>. It should be interesting, therefore, to ascertain whether the dominant clonotypes observed in adult responses, for example, the TEPC 15 clonotype specific for phosphorylcholine, are expressed as neonatal predominant clonotypes, and thus fulfil another criterion for 'germ line' representation. In this report, the frequency of phosphorylcholine-specific B cells is quantitated in neonatal BALB/c mice. The results indicate that phosphorylcholine-specific clonal precursor cells, even those of the dominant TEPC 15 clonotype, appear quite late in neonatal development.

Spleen cells from BALB/c mice at various ages from birth to adulthood were transferred intravenously to lethally irradiated (1,300-1,600 r.) adult BALB/c mice immunised 2-3 months previously with 0.1 mg of *Limulus polyphemus* haemocyanin (Hy)<sup>2</sup>. Sixteen hours after transfer, spleen fragment cultures were prepared from recipients as before<sup>2</sup>, and cultures were stimulated *in vitro* for 3-4 d with either 1×10<sup>6</sup> M DNP-Hy or 5×10<sup>7</sup> M 3-(p-azo-phenylphosphorylcholine) - N - acetyl - L - tyrosylglycylglycine - haemocyanin (PPC-TGG-Hy), an antigen shown to maximise carrier recognition in anti-phosphorylcholine responses<sup>6</sup>. Culture fluids collected from day 9 to day 13 after culture initiation were analysed for the anti-phosphorylcholine or anti-DNP antibody by a sensitive radioimmunoassay. The binding of antibody to the antigen coupled to bromoacetyl cellulose immunoabsorbent was detected with <sup>125</sup>I-labelled rabbit anti-mouse Fab antibody<sup>2,6</sup>. Previous studies have demonstrated the donor origin of the antibody-producing cells<sup>16</sup>, the monoclonal nature of the antibody obtained<sup>2,12,16</sup>, the efficiency of detecting clonal precursors in this system (3.9%) (ref. 16 and unpublished results of A. Pickard and N.R.K.), and the efficiency of such stimulation for the analysis of neonatal B cells<sup>3,12,13,16</sup>. Antibody with the TEPC 15 idiotype was detected in an inhibition solid-

Table 1 Number of phosphorylcholine-specific foci and percentage of those foci positive for the TEPC 15 idiotype at various ages

Age (d)	Total No. of neonates	Total No. of cells analysed	No. of foci	% Foci with TEPC 15 idiotype
0-2	53	148 × 10 <sup>6</sup>	0	—
3-5	15	66 × 10 <sup>6</sup>	0	—
6-7	34	628 × 10 <sup>6</sup>	26	66
8-9	22	320 × 10 <sup>6</sup>	13	77
14	26	823 × 10 <sup>6</sup>	56	72
21	6	231 × 10 <sup>6</sup>	24	57
28	3	199 × 10 <sup>6</sup>	25	72



**Fig. 1** The frequency of clonal precursor cells specific for phosphorylcholine or DNP at various ages. Two to  $25 \times 10^6$  donor spleen cells from conventionally reared mice were transferred into an individual recipient, and the recipient spleen fragments were stimulated with PPC-TGG-Hy or DNP-Hy. Each small circle represents the phosphorylcholine-specific precursor cell frequency derived from one to ten donor spleens injected into two to eight Hy-primed recipients, the bars indicate the average frequency at each age. The adult precursor cell frequency for phosphorylcholine is derived from data published before<sup>11</sup>. Since no phosphorylcholine-specific foci were detected in 2- and 4-d-old donors, the bars at those ages denote the maximum precursor frequency if one focus had been detected. The open circles represent the average DNP-specific precursor cell frequency at various ages; the data have been published for neonates<sup>13</sup> and adults<sup>2, 10</sup>. The closed boxes indicate the average frequency and standard deviation of single DNP-specific predominant clonotypes, assuming that each of the three predominant clonotypes is represented equally in the early neonatal period. All the data are represented as the frequency of clonal precursor cells in  $10^6$  spleen cells, using a cloning efficiency of 3.9% as the correction factor<sup>10</sup>.

phase radioimmunoassay using anti-TEPC 15 sera raised in A/He mice<sup>6</sup>.

Table 1 shows that of the 53 neonates examined from birth to 2 d of age and the 15 neonates analysed from 3 to 5 d of age, no foci producing anti-phosphorylcholine antibody were detected. The first phosphorylcholine-specific clonotypes arise approximately 1 week after birth, and most of these clones are of the TEPC 15 idotype, as is the case in the BALB/c adult<sup>6</sup>. Figure 1 plots the frequency of DNP and phosphorylcholine-specific precursor cells in adults and neonates during the first several weeks of life. As reported previously, DNP-specific cells are present in high frequency at birth and remain relatively constant into adulthood<sup>12</sup>. Conversely, phosphorylcholine-specific precursor cells do not appear at all until day 6–7 after birth and remain in relatively low frequency for several weeks. Although, as Fig. 1 demonstrates, both conventionally reared neonates and adults show wide variations in phosphorylcholine-specific precursor frequency, the average neonatal frequency at 1 week old is eight times lower than the average adult precursor cell frequency.

The DNP-specific precursors present in the spleen during the first 5–6 d after birth are largely representative of only three predominant clonotypes<sup>13</sup>. A DNP-specific B cell represents one of 3,000 total B cells, therefore, the frequency of any one of the predominant clonotypes is one in

$10^4$  B cells. This is the same frequency observed for the three TNP-specific predominant clonotypes, which have been shown to be distinct from those specific for DNP by isoelectric focusing<sup>13</sup>. Thus while the frequency of each of the six known neonatal predominant clonotypes is one in  $10^4$  B cells, the data presented here indicate that early in development the TEPC 15 clonotype is 100 times less frequent than clonotypes presumably representing the earliest expression of the germ line genetic information for antibody specificities.

An analysis of 26 germ-free adults from various sources shows a frequency similar to conventionally reared adults, so that it is unlikely that antigen contact plays a significant role in either the generation of precursors with the TEPC 15 idotype or the maintenance in high numbers in adult mice<sup>11</sup>. Preliminary studies using germ-free neonates 5 and 8 d old demonstrate that the kinetics of appearance of phosphorylcholine-specific precursors in germ-free neonates is, in general, similar to that seen in conventionally reared mice (our unpublished results). Although neither maternal influences nor the presence of antigen can be ruled out specifically as the reason for the late acquisition of the TEPC 15 clonotypes during the neonatal period, the preliminary data with the germ-free neonates suggests that this is not the case.

Since the TEPC 15 clonotype is a prototype of a 'germ line' specificity, its late appearance during ontogeny may have significant implications for the mechanism by which the B-cell repertoire is acquired. This report and others have shown that the generation of the repertoire seems to be antigen independent<sup>3, 12, 13, 16</sup>, and the findings reported here indicate that the expression of even ubiquitous clonotypes may be relatively late events in neonatal development.

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## Classical pathway activation of complement system by IgA anti-C3 antibody

ANTIBODIES which react with native C3 are present in high titre in adult<sup>1</sup> and neonatal saliva<sup>2</sup>. Although they belong to the IgA class they are macromolecules with a sedimentation coefficient of greater than 19S. They are also unusual in that their binding to C3 is dependent on the presence of

Table 1 C3 conversion induced by neonatal anti-C3 antibody

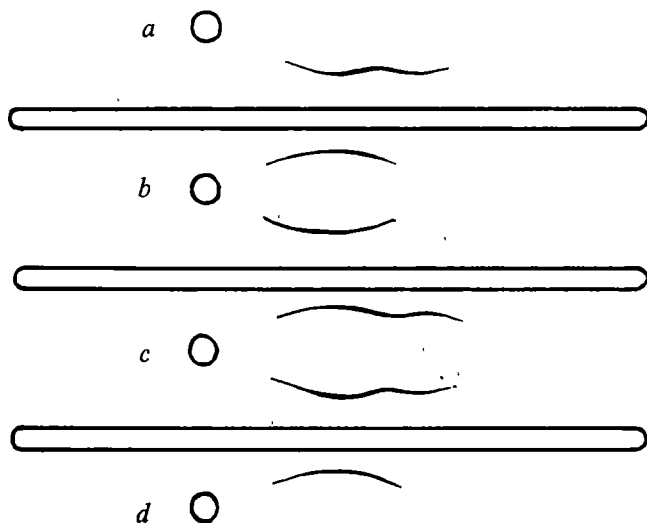
	EDTA plasma (%)	EGTA plasma (%)	Serum (%)	C2 Deficient serum (%)
IgG (50 µg)	< 5	< 5	38	5
Zymozan	< 5	31	36	23
Anti-C3 1:5	< 5	5	31	< 5
Anti-C3 1:10			27	7
Anti-C3 1:20			17	< 5

calcium ions and they can be dissociated from C3 by 0.01 M EDTA<sup>1,2</sup>. Our preliminary studies on the properties of this antibody had shown that it could activate the complement system and in this paper we report investigations to determine whether this proceeds by way of the classical or alternative pathway.

Activation of the complement system was assessed by measuring the conversion of C3 occurring in normal human serum after adding anti-C3 antibody, the amount of conversion being calculated by planimetry<sup>3</sup>. Dilutions of purified antibody<sup>2</sup> were added to equal volumes of fresh human serum, EDTA plasma, EGTA plasma and C2-deficient human serum, and then incubated at 37 °C for 45 min. Heat-aggregated IgG and zymozan were included as positive controls. The results obtained are summarised in Table 1. Both the aggregated IgG and the zymozan produced C3 conversion in normal serum. The aggregated IgG did not convert C3 in EGTA plasma or C2 deficient human serum whereas zymozan produced C3 conversion in both sera. Conversion of C3 occurred when purified anti-C3 antibody was added to human serum and the amount of conversion was proportional to the amount of antibody added. No conversion of C3 occurred when antibody was added to C2-deficient human serum or EGTA plasma. C3 conversion in normal guinea pig serum and C4-deficient guinea pig serum was assessed qualitatively after immunoelectrophoresis in 1% agar, diluted in barbitone buffer pH 8.6 containing 0.01 M EDTA. The antibody, although capable of converting C3 in normal guinea pig serum, failed to convert C3 in C4 deficient serum (Fig. 1).

The ability of the antibody to activate C1 directly was

Fig. 1 Results of immunoelectrophoresis showing conversion of C3 in normal guinea pig serum (GPS) but no conversion in C4 deficient guinea pig serum (C4D). a, C4D and zymozan; b, C4D and anti-C3 antibody; c, GPS and anti-C3 antibody; d, GPS, EDTA to 0.01 M and anti-C3 antibody. Trough contains goat anti guinea pig C3 (Nordic Diagnostics).



then examined in serum from a patient with hereditary angioneurotic oedema which contained only 10% of the normal concentration of C1 inhibitor. Anti-C3 antibody (100 µl) was added to 800 µl of complement buffer, 100 µl of serum and 50 µl of 1 M acetyl tyrosine ethyl ester and then incubated with constant stirring at 37 °C. C1 was measured enzymatically by the hydrolysis of the ester<sup>4</sup>. When antibody was excluded from the mixture very little spontaneous activation of C1 took place, but the addition of purified antibody or heat aggregated IgG resulted in rapid activation of C1.

The observations that anti-C3 antibody in combination with antigen can activate C1 directly and that it fails to convert C3 in C2 and C4 deficient serum indicates that complement activation requires the early components C1, C4 and C2. In man, previous reports have shown that complexes or aggregates of IgA activate the complement system via the alternative pathway<sup>5</sup>. Our studies on the salivary anti-C3 antibodies show for the first time that IgA antibody can also activate the complement system via the classical pathway. This pattern of activation may account for the observations that the haemolytic titres of C3 and C4 are low and that inactivated forms of these two proteins are present in colostrum<sup>6</sup>, this secretion being another source of the anti-C3 antibody in humans<sup>7</sup>. Since these antibodies are macromolecular in size and have a number of unusual properties our findings may not be applicable to other IgA antibodies.

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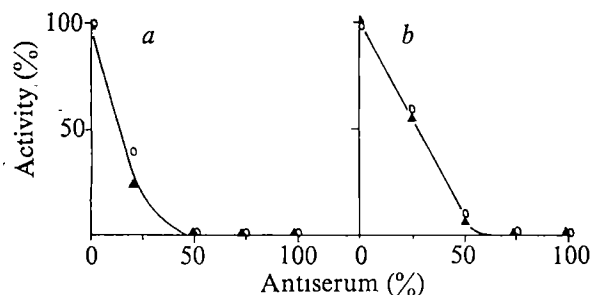
## Antibodies against homogeneous epoxide hydratase provide evidence for a single enzyme hydrating styrene oxide and benz(a)pyrene 4,5-oxide

THE microsomal enzyme epoxide hydratase (EC 4.2.1.63) is potentially important in the inactivation of metabolically produced epoxides which may be responsible for the mutagenic and/or carcinogenic properties of polycyclic hydrocarbons (for reviews see refs 1-3). Reports<sup>4,5</sup> suggest that the enzyme plays a dual role in (a) producing proximate carcinogens which, after biotransformation to carcinogens by microsomal mono-oxygenase(s) are (b) inactivated by epoxide hydratase. As this enzyme can be induced<sup>6-8</sup>, activated<sup>9-10</sup> and inhibited<sup>9-13</sup> it should be useful in studies of the mechanism of chemical carcinogenesis: some inverse correlations have been reported between susceptibility to carcinogenesis by polycyclic hydrocarbons and inducibility of epoxide hydratase at different stages of development in the rat<sup>14</sup>. Most investigations of this enzyme<sup>6-10,15-18</sup>, however, have involved radiometric assay with <sup>3</sup>H-styrene oxide as substrate<sup>18</sup>. There is evidence that the enzyme(s) responsible for the hydration of this substrate are also involved in hydration of epoxides, derived

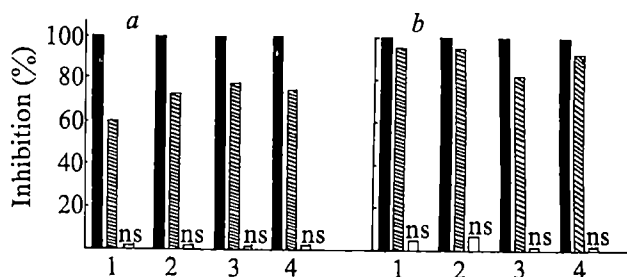
from polycyclic hydrocarbons<sup>9,18</sup>, while other evidence suggests the presence of several epoxide hydrolases in the microsomal membrane<sup>7,9,10,19</sup>. Thus it was not known whether information about epoxide hydrolase obtained with styrene oxide as substrate applied to the enzyme(s) responsible for hydration of epoxides derived from polycyclic hydrocarbons. Using antibodies raised against a homogeneous epoxide hydrolase preparation and other criteria described here, we have now found evidence that intact microsomal membranes contain a single enzyme able to hydrate styrene oxide and benz(a)pyrene, 4,5-oxide, a K-region epoxide.

Epoxide hydrolase was solubilised from adult male Sprague-Dawley rat liver microsomes and purified to homogeneity as established by gel electrophoresis, analytical ultracentrifugal and immunological criteria<sup>20</sup>. Antibodies to this preparation were raised in New Zealand white rabbits. The animals were immunised intradermally at several sites along the flank with 100 µg of pure epoxide hydrolase in Freund's complete adjuvant. Booster injections of 50 µg of protein in 50 mM Na phosphate buffer were given intradermally 8 and 16 weeks later. Rabbits were bled and serum was collected 8–10 d after the last injections. A 200 µl sample of either crude epoxide hydrolase solution (solubilised microsomes, 1.4 mg protein) or homogeneous epoxide hydrolase (46 µg) was kept for 18 h at 0–4 °C in the presence of a constant amount (200 µl) of serum containing various amounts of antiserum. Controls were kept in the same conditions but in the presence of control serum. Precipitated antigen-antibody complexes were sedimented by centrifugation at 8,000g for 20 min and supernatant fractions were assayed for epoxide hydrolase activity in the absence of Tween 80 with substrates styrene oxide<sup>10</sup> and benz(a)pyrene 4,5-oxide (H. U. Schmassmann *et al.*, unpublished). To determine the inhibitory potency of the antibodies, enzyme preparations were kept in the presence of antiserum for 15 min at room temperature and then assayed without previous centrifugation. In the experiments with low molecular weight inhibitors, these were added to the incubation mixture at zero time with no preincubation, in 10 µl of acetonitrile with styrene oxide and in 25 µl of acetonitrile with benz(a)pyrene 4,5-oxide as substrates, while controls had the same amounts of acetonitrile.

Figure 1 shows the results of an immunoprecipitation



**Fig. 1** Immunoprecipitation of epoxide hydrolase activity with styrene oxide (▲) and benz(a)pyrene 4,5-oxide (○) as substrate in crude hydrolase solution (solubilised microsomes) (a), and homogeneous hydrolase (b) by antibodies raised against an epoxide hydrolase preparation homogeneous by gel electrophoresis, analytical ultracentrifugal and immunological criteria. The results are expressed as percentage of the activity of samples treated similarly with serum from control animals. These control activities were 10.6 and 432 nmol styrene glycol and 3.7 and 136 nmol benz(a)pyrene 4,5-dihydrodiol per min per mg protein with the crude hydrolase solution and the homogeneous hydrolase, respectively. Differences between these controls and samples without serum protein were always less than 30% and were noticed only when homogeneous epoxide hydrolase was used, probably because the protein content present in those assay mixtures was extremely low in the absence of serum proteins.



**Fig. 2** Inhibition of epoxide hydrolase activity with 2 mM styrene oxide (a) and 0.15 mM benz(a)pyrene 4,5-oxide (b) as substrate by 2 mM 1,1,1-trichloropropene 2,3-oxide (■), 2 mM cyclohexene oxide (▨) and 2 mM *trans*-stilbene oxide (□). Enzyme preparations were: 1, rat liver microsomes; 2, solubilised microsomes; 3, phosphocellulose column effluent; 4, homogeneous epoxide hydrolase. The specific activities of the various preparations reflecting their relative purification were: 1, 5.01; 2, 8.91; 3, 260; 4, 600 nmol styrene glycol per min per mg protein. Inhibitors were added at zero time in acetonitrile solution. Controls received the same amount of acetonitrile. Results are expressed as percentage inhibition compared with such controls. ns, not significant (level of significance:  $P < 0.05$ ).

experiment. Antibodies raised against homogeneous epoxide hydrolase, which had been purified<sup>20</sup> using an assay with styrene oxide as substrate<sup>10</sup>, precipitated epoxide hydrolase activity towards both styrene oxide and benz(a)pyrene 4,5-oxide. Precipitation, which was dose-dependent, occurred when antiserum was incubated with either the pure enzyme or the crudest soluble preparation (solubilised microsomes). Immunoprecipitation was complete at the higher concentrations of antiserum. This suggested the presence of a single enzyme responsible for the hydration of the two substrates. On the other hand, there may be several immunologically cross reactive epoxide hydrolases or some epoxide hydrolase species may be inactivated differentially but completely during solubilisation of the microsomes. But although the extent of inhibition of epoxide hydrolase activity by antiserum was different in microsomes (20–30%) compared with soluble preparations (70–80%), inhibition of the hydration of the two substrates was always similar ( $\pm$  less than 10% of the mean) (data not shown). Furthermore, the relative potency of low molecular weight epoxide hydrolase inhibitors remained essentially the same throughout purification. This was true for either substrate. As Fig. 2 shows, when styrene oxide was used as substrate the membrane-bound enzyme (1), the crudest solubilised preparation (2), a partially purified preparation (3), and homogeneous epoxide hydrolase (4), were all inhibited completely by 1,1,1-trichloropropene 2,3-oxide, very strongly ( $\sim$  60–80%) by cyclohexene oxide and not significantly ( $< 10\%$ ) by *trans*-stilbene oxide. Epoxide hydrolase activity with benz(a)pyrene 4,5-oxide as substrate was also inhibited by these agents to very similar extents in the different enzyme preparations. Finally, only a single side fraction during purification had detectable epoxide hydrolase activity towards either of the substrates used. The ratio of the specific activities towards the two substrates was the same in this single side fraction as in the fraction containing the bulk of epoxide hydrolase activity.

These results, taken together, strongly suggest that a single enzyme is involved in the hydration of both styrene oxide and benz(a)pyrene 4,5-oxide in rat liver microsomes. Thus, information already acquired about epoxide hydrolase, using styrene oxide as substrate, seems to be directly relevant to the enzymatic entity responsible for hydration of benz(a)pyrene 4,5-oxide, a K-region epoxide and, most probably, many epoxides derived from several carcinogenic polycyclic hydrocarbons.

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## Complexity of germline and somatic DNA in *Ascaris*

DURING the early determination of the blastomeres in the nematode *Ascaris* chromosomal segments are eliminated in the pre-somatic cells (chromatin diminution). In the germline, however, all the chromatin is preserved. Similar genome differentiation phenomena have been published<sup>1–3</sup>. Since no molecular description of chromatin diminution has been made we have begun to study the chromosomes in germline and somatic cells<sup>4</sup>. It was found that the amount of DNA occurring only in the germline chromosomes is not constant<sup>5</sup> and that this DNA is not enriched in rDNA<sup>6</sup>. There is no evidence that the germline-limited DNA is transcribed *in vivo*<sup>7</sup>. The results presented here show that the germline DNA of *Ascaris* contains highly repetitive components, eliminated by the diminution process. Their possible molecular function in connection with germline-soma differentiation and chromosomal behaviour is discussed.

By means of Feulgen photometric measurements and Burton's reaction, we determined the DNA content of germline and soma genomes of *Parascaris equorum* race *univalens* as 1.2–2.1 pg and 0.25 pg, and *Ascaris lumbricoides* var. *suis* as 0.32 pg and 0.25 pg, respectively. Therefore, *Parascaris* contains up to 85% germline-limited DNA but *A. suis* only 22%. An analysis of melting curves had shown that the G+C content of the germline DNA differs from that of soma DNA<sup>8</sup>. Buoyant density profiles of DNA in neutral CsCl gradients demonstrate the presence of germline satellites in both species (Fig. 1). Germline DNA of *P. equorum* contains two satellites of sizes unique in the animal kingdom, with densities 1.697 g cm<sup>-3</sup> and 1.692 g cm<sup>-3</sup> (Fig. 1a); the dense shoulder at 1.700 g cm<sup>-3</sup> represents the single copy DNA. After denaturation and reassociation to low *C<sub>0</sub>t* (Fig. 1a') the highly repetitive components (satellites) form one peak at 1.703 g cm<sup>-3</sup>, making up about 85% of the total germ-

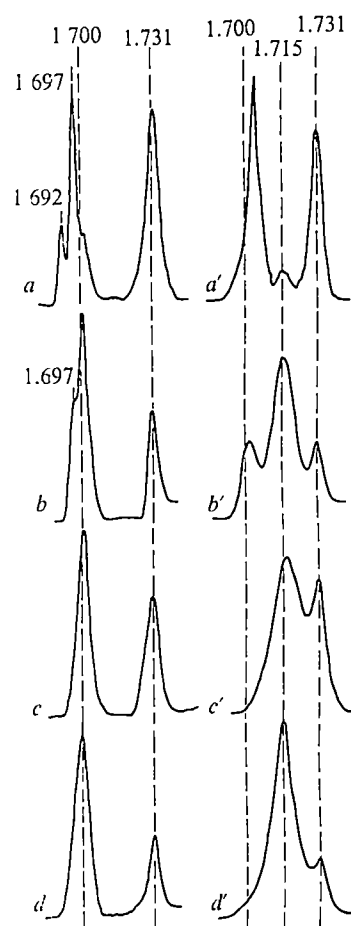


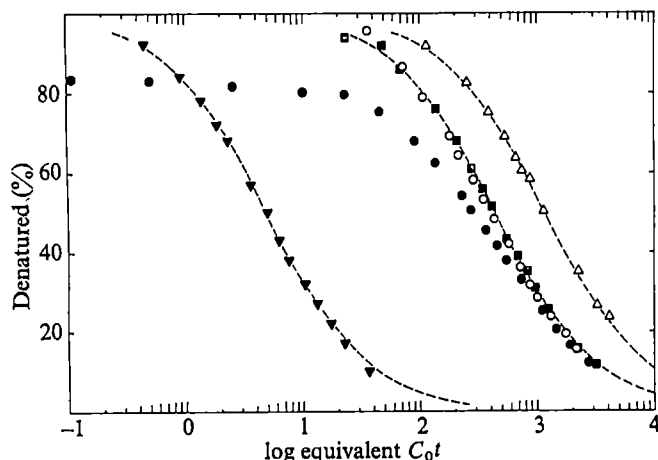
Fig. 1 Buoyant density pattern of *Ascaris* DNA centrifuged in neutral CsCl. Left column, native DNA; right column, denatured unsheared DNA reassociated to *C<sub>0</sub>t* 0.5 mol nucleotide s<sup>-1</sup>. a, a', Spermatid germline DNA of *Parascaris equorum* race *univalens*; b, b', spermatid germline DNA of *Ascaris lumbricoides* var. *suis*; c, c', larval soma DNA of *P. equorum*; d, d', larval soma DNA of *A. suis*. Marker DNA was from *Micrococcus lysodeikticus* with density taken as 1.731 g cm<sup>-3</sup>, implying an *E. coli* DNA density of 1.710 g cm<sup>-3</sup> in our analytical centrifugations. Mitochondrial DNA of *Ascaris* bands at 1.686 g cm<sup>-3</sup> (ref. 11). For further explanation see text.

line DNA. The single copy DNA, still denatured, forms the side peak at 1.715 g cm<sup>-3</sup>. The sharp profile of the reassociated fraction may indicate short basic nucleotide sequences of the satellite DNA. Since only one peak is generated one might deduce that there is a sequence relationship between the two satellites. In *A. suis* the satellite DNA at 1.697 g cm<sup>-3</sup> (Fig. 1b) is not sharply separated from the main band DNA as reported earlier<sup>9</sup>. After denaturation and reassociation to low *C<sub>0</sub>t*, however, the single-stranded DNA has shifted away from the reassociated satellite DNA (Fig. 1b'), which is considered to be about 23% of the germline DNA. In the native larval somatic DNA of both species (Fig. 1c and d) satellites are absent. Apparently they have been lost during chromatin diminution. Even after density transposition of single copy DNA by denaturation and following low *C<sub>0</sub>t* reassociation there is no evidence for satellite DNA (Fig. 1c' and d'). If we incubate denatured unsheared soma DNA of *A. suis* to low *C<sub>0</sub>t* and subsequently digest it by single-strand specific endonuclease, 4% is revealed as enzyme resistant on CsCl centrifugation.

Reassociation kinetics (Fig. 2) of total genome DNA of *A. suis* demonstrate that 17.5% of testis DNA (predominantly germline DNA) consists of repeated sequences whereas the larval soma DNA does not contain any measurable highly repetitive component. This latter finding contradicts the data of Tobler *et al.*<sup>10</sup> who reported a value of 10% repetitive DNA

for soma DNA. In spermatid DNA which is not contaminated by follicle epithelium DNA and contains only insignificant amounts of mitochondrial DNA repetitive sequences make up 20–22% (refs 8 and 10).

The slowly-reassociating DNA of germline and of soma reassociates homogeneously with second order kinetics (Fig. 2), and the reassociation rate constants correspond well. This indicates that there is identical single copy DNA complexity in the germline and the soma. The  $C_0t_{1/2}$  values corrected for 50% G+C (ref. 11) suggest that the somatic DNA as well as the plurivalent germline chromosomes constitute the unit genome<sup>12</sup> since their analytical values compare well with the reassoci-



**Fig. 2** Reassociation kinetics, analysed optically, of larval soma DNA (■), of total testis (germline) DNA (●) and single copy testis DNA (○) of *A. suis*, of *E. coli* DNA (▼) as a standard and of single copy DNA of *Gallus domesticus* (△). Larval DNA was extracted from embryos, testis DNA from whole gonads. The DNA isolation involved proteinase K (Merck) digestion of the lysates, deproteinisation by phenol, pancreatic RNase and T<sub>1</sub> RNase digestion; and hydroxyapatite column chromatography. DNA was sonicated in 0.01 M Tris-HCl pH 7.1 to a mean fragment size of 400 nucleotide pairs. Reassociation buffer was 0.01 M Tris-HCl, 2 M NaClO<sub>4</sub> pH 7.1. After melting of the DNA in a water-jacketed cuvette (pathlength 1 mm) the temperature was lowered within seconds to incubation temperature (25 °C beneath  $T_m$ ). The absorbance was recorded by means of a double beam equipment. Timed absorbance values were stored with an interface in a computer. For the description of the second order reaction the following equation was taken

$$(\Delta_t^{\text{ren}})^{-1} = (\Delta_0^{\text{ren}})^{-1} + kt$$

$$\text{with } \Delta_t^{\text{ren}} = A_t - A_{\infty}^{\text{ren}} \\ \Delta_0^{\text{ren}} = A_0 - A_{\infty}^{\text{ren}} \\ k = (\Delta_0^{\text{ren}} \times t_{1/2})^{-1}$$

$A_t$  is the absorbance at time  $t$ ,  $A_0^{\text{ren}}$  that after deducting collapse hypochromicity<sup>13</sup>,  $A_{\infty}^{\text{ren}}$  that for infinite reaction, which is approximated by linear regression for the time interval within which 75% of the single copy DNA has reassociated. From the regression line the  $C_0t$  curves were obtained using:

$$\Delta_t^{\text{ren}} \times (\Delta_0^{\text{ren}})^{-1}$$

as fraction of denatured DNA.  $C_0t$  was transformed to  $Ec_0t$  (ref. 16) (incubation buffer 0.12 M phosphate). In 2 M NaClO<sub>4</sub> reassociation is 5.5 times faster than in 0.12 M phosphate. The dashed lines are theoretical second order curves. For *E. coli* DNA  $Ec_0t_{1/2} = 4.8$ . The germline DNA from testis contains 17.5% fast reassociating sequences. The  $C_0t$  curve of germline single copy DNA ( $Ec_0t_{1/2} = 410$ ) was evaluated from the curve of total testis DNA. For larval soma DNA  $Ec_0t_{1/2} = 440$ . For single copy chicken DNA  $Ec_0t_{1/2} = 1,190$ . DNA concentrations were 461  $\mu\text{g ml}^{-1}$  for *E. coli*, 772  $\mu\text{g ml}^{-1}$  for *Ascaris* testis, 1,172  $\mu\text{g ml}^{-1}$  for *Ascaris* larva, and 862  $\mu\text{g ml}^{-1}$  for *Gallus*.

**Table 1** Comparison of genome sizes and kinetic complexity in daltons

DNA (% G+C)*	Analytical value	Kinetic value†
<i>E. coli</i> (50)	$2.7 \times 10^9$	
<i>A. suis</i> soma (40.8)	$1.5 \times 10^{11}$	$1.9 \times 10^{11}$
<i>A. suis</i> germline (40.8)	$1.9 \times 10^{11}$	$2.2 \times 10^{11}$
<i>Gallus domesticus</i> (42)	$7.5 \times 10^{11} \ddagger$	$5.9 \times 10^{11}$

\*G+C content of single copy DNA.

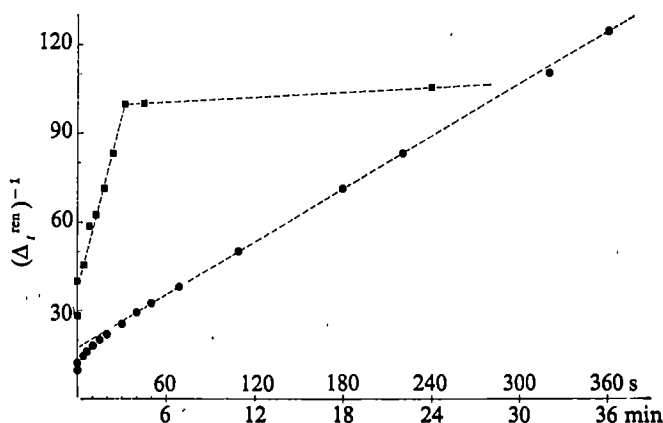
†Corrected for G+C content and proportion of fast reassociating components.

‡Ref. 13.

ation kinetic values using *Escherichia coli* DNA as a standard, within the limitations of the method (Table 1). Consequently, in the germline genome there is no room for any appreciable amount of germline-limited single copy DNA (estimated to be one half of germline-limited DNA by ref. 10), which could be detectable by means of reassociation kinetics studies. This is independently confirmed by the agreement of the two percentages; the germline-limited DNA (22%) and the fast-reassociating DNA fraction (20–22%) of the spermatid (that is, germline) genome. If one considers both the cytology of diminution and that the soma DNA does not contain satellite DNA then the identity of those two percentages shows that the reiterated sequences are clustered.

We have repeatedly measured the reassociation of the more complex single copy DNA of *Gallus domesticus*. Using *E. coli* DNA as a standard, the  $C_0t_{1/2}$  value was always estimated to be

**Fig. 3** Reassociation, measured by hypochromicity, of isolated highly repetitive components of soma and germline DNA of *A. suis*. Sheared larval soma DNA and testis DNA were denatured and incubated to  $Ec_0t = 3$ . The reassociated DNA was bound on to a 60 °C hydroxyapatite column equilibrated in 0.12 M phosphate, the absorbed fraction eluted by 0.35 M phosphate washing. Soma DNA yields a bound fraction between 3.5–6.5%, testis DNA about 14%. Reassociation of fast reassociating sequences was done in 0.03 M phosphate, where reassociation is 75 times slower than in 0.12 M phosphate<sup>16</sup>. Reassociation was recorded in double beam using water-jacketed cuvettes (10 mm pathlength) and temperature measuring equipment in the reference cuvette. In the experiment shown, the soma DNA fraction (■), contaminated by single copy DNA, renatured to 55%, of which 35% (2.3% of total soma DNA) occurred within 30 s. For this fraction  $\Delta_0^{\text{ren}}$  was 0.16, hence at  $Ec_0t = 10^{-5}$  this fraction (foldback DNA) had completely renatured. The other reassociating fraction makes up 20% (1.3% of total soma DNA). The reassociation of the highly repetitive testis DNA (●) may be interpreted as the reaction of three components: one reassociating with  $t_{1/2} = 5.7$  min and  $\Delta_0^{\text{ren}} = 0.057$ , thus yielding an  $Ec_0t_{1/2}$  of  $2 \times 10^{-4}$ , making up some 70% of the fast reassociating sequences. The additional 30% ( $\Delta_0^{\text{ren}} = 0.024$ ) contain 13% (2.3% somatic foldback DNA correspond to 1.8% in the germline; 1.8%:14% foldback DNA and 17% highly repetitive sequences. For symbols see legend of Fig. 2. Abscissa: time of incubation; in s for soma DNA, in min for germline DNA.



smaller than expected from the analytical genome size for which divergent values have been published, however.

In order to characterise the repeated DNA sequences, they were isolated from total DNA. Reassociation to low  $C_0t$  of denatured sheared total larval soma DNA of *A. suis* (Fig. 3) yields a renatured fraction of approximately 4%. This was isolated as 0.35 M phosphate buffer fraction by HAP column chromatography using 0.12 M phosphate as the discriminating concentration between single and double-stranded fragments. The proportion of the fast-renaturing DNA is 2.3% of the total DNA. It has completely renatured at  $Ec_0t < 10^{-5}$ , and has a sharp melting profile. Its hyperchromicity, however, is low. Its broad banding profile in neutral CsCl reveals its network configuration; it is also highly resistant to endonuclease. These are all characteristics of foldback DNA<sup>14</sup>. Continued reassociation, after the foldback DNA has completely renatured, reveals a slowly reassociating component which is about 1% of the total DNA. Its percentage is small compared with that found in other organisms. If one accepts that intermediate repetitive sequences have a regulatory function<sup>15</sup>, the small amount present in *Ascaris* reflects the simple organisation of this organism.

From denatured sheared testis (that is, germline) DNA after reassociation to low  $C_0t$  the reassociated fraction isolated by HAP chromatography makes up 14% of the total DNA. Its reassociation indicates fast reassociating sequences in addition to the ubiquitous foldback DNA. Their reassociation curve (Fig. 3) may be interpreted as the reaction of three components: the foldback DNA and two highly repetitive classes of DNA: One makes up 21% of the germline-limited DNA which reassociates with  $C_0t_{1/2} < 10^{-4}$ , the other predominant one (79%) reassociates with  $C_0t_{1/2} = 2 \times 10^{-4}$ . Alternatively, the initial delay of the reaction, indicated by the continuous decline of the slope of the curve in Fig. 3, may be caused by increasing hyperpolymerisation<sup>16</sup> during the reassociation of only one component degenerated in its basic nucleotide sequence. In neutral CsCl, the isolated germline-limited DNA forms one sharp peak. This, together with its high hyperchromicity, indicates its highly native state after reassociation. Its  $T_m$  is 3 °C lower than that of the foldback DNA.

The observations on the abnormal cleavage of the *Ascaris* egg<sup>17</sup> have shown that the existence of germline limited chromatin is a sufficient criterion of the germline quality of a blastomere; the irradiation of germline blastomeres<sup>18</sup> showed that the existence of that chromatin is necessary. The data presented here demonstrate, for the first time for an organism with germline-limited chromatin, that the presence of highly repetitive DNA components in itself is sufficient for the genetic status of a germline cell. Yet, in spite of the developmental significance of this type of chromatin, the molecular function of its highly repetitive DNA components remains obscure.

But three properties of the *Ascaris* germline chromosomes may be related to this function. (1) The highly repetitive DNA sequences are components of the heterochromatic sections of the germline chromosomes. After diminution the chromosomes are euchromatic. Therefore, these sequences might possibly suppress the activity of somatic genes, as happens in position effect variegation, and thus be responsible for the maintenance of the germline quality. (2) Germline chromosomes have a tendency to form plurivalent chromosomes. In extreme cases, the entire genome is integrated into a single plurivalent chromosome. The tandem reiteration of distally localised sequences allows the formation of sticky ends and, consequently, the linking together of chromosomes. Aggregation of the minute somatic single chromosomes to large plurivalent chromosomes prevents mistakes in meiotic distribution. (3) The germline chromosomes are holocentric<sup>4</sup>. The germline-limited chromatin has kinetic activity in anaphase. This property, which is lost in preparation for diminution, increases the chromosome manoeuvrability. This latter must be guaranteed, especially in parasitic organisms such as *Ascaris* which produce exceedingly large quantities of gametes. These observations in *Ascaris* and

their interpretations support quite well Walker's ideas<sup>19</sup> on the significance of highly repeated sequences.

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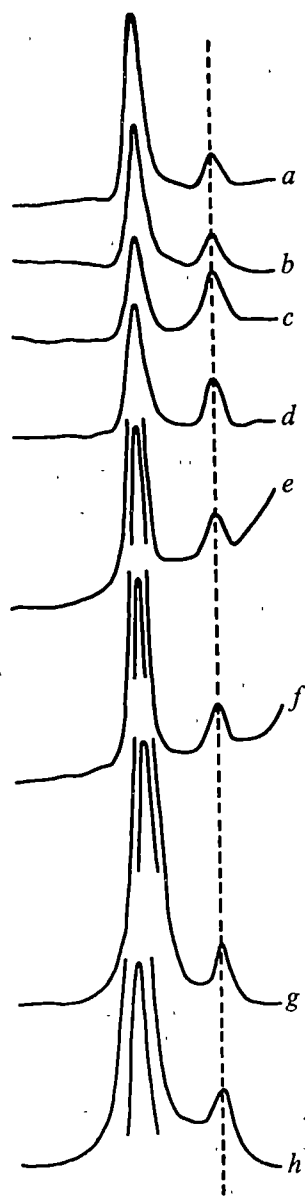
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## No detectable satellite DNA in supernumerary chromosomes of the grasshopper *Myrmeleotettix*

SUPERNUMERARY (B) chromosomes and satellite (simple sequence) DNA are enigmatic chromosomal phenomena, in that both are variable additions to the main chromosome complement. It has been suggested that differences between, and constancy within, certain related species for satellite DNA, implies that it may play a role in speciation by a series of saltatory steps<sup>1-3</sup>. Similarly, B chromosomes may affect recombination<sup>4</sup> or rate of development<sup>5</sup>, or bring about diploidising effects in hybrids of closely related species<sup>6</sup>. There is still, however, no clear understanding of the functions of either of these variables.

The first report of between and within-population variation in satellite DNA, associated with the presence or absence of B chromosomes in the grasshopper *Myrmeleotettix maculatus*<sup>7,8</sup>, suggested that there might be a closer relationship between satellite DNA and supernumerary chromosomes. Analysis of sedimentation patterns of total DNA in neutral caesium chloride showed that the DNA of B chromosome-containing individuals, from several different populations, contained satellite DNA not found in individuals lacking B chromosomes. Furthermore, both main-band and satellite DNA were found to vary in buoyant density in populations from different localities.

Several studies, however, on the heterogeneity of B chromosome DNA in plants<sup>9-11</sup>, have failed to reveal large fractions of highly repetitive or moderately repetitive DNA exclusive to the B chromosomes. Detailed examination of several parameters of base composition of A and B chromosome DNAs were made in the wheat group of species<sup>11</sup>. These included profiles of thermal dissociation of homologous and heterologous duplexes, differential melting curves in strictly comparable den-



**Fig. 1** Analytical ultraviolet absorbance patterns, after sedimentation to equilibrium in neutral CsCl for 20 h at 44,000 r.p.m. at 25 °C on a Centriscan 75 analytical centrifuge. *a-f*, Pronase-digested lysates of testes or ovaries of single individuals. Tissue was lightly ground in glass homogenisers in 150  $\mu$ l of 0.5% sodium lauryl sulphate, 0.1 M EDTA, 0.05 M Tris, pH 8.4 and lysed for 2 h at room temperature. After lysis, 0.5 ml of 10 mM Tris-HCl, pH 7.8 and 100  $\mu$ l of self-digested Pronase (1 mg ml<sup>-1</sup>) were added and incubated for 2 h at 37 °C. Solid CsCl (ultra-centrifuge grade) was added to an initial density of 1.700 g cm<sup>-3</sup>. A sample of 2  $\mu$ l of marker DNA ( $A = 4$ ) of *Pseudomonas aeruginosa* (density = 1.726 g cm<sup>-3</sup>) was added. *g-h*, Bulk testes of 25 individuals were homogenised in 4 M CsCl (initial density = 1.4 g cm<sup>-3</sup>) in a glass homogeniser. The homogenate was centrifuged in a 8  $\times$  14 ml rotor of an MSE 65 centrifuge for 20 h at 40,000 r.p.m. at 25 °C. The pellet was dissolved in 4 ml of 1  $\times$  SSC (0.15 M NaCl + 0.015 M Na citrate) and incubated at 37 °C successively with  $\alpha$ -amylase (250  $\mu$ g ml<sup>-1</sup>) for 1–2 h, RNase (250  $\mu$ g ml<sup>-1</sup>, preheated to 80 °C for 5 min) for 1–2 h, and Pronase (1 mg ml<sup>-1</sup>, self-digested at 37 °C for 2 h) for 1–2 h. Insoluble material was removed with low speed centrifugation and the DNA was pelleted for 1–2 h at 40,000 r.p.m. at 4 °C and dissolved in 10 mM Tris-HCl, pH 7.8. Conditions for analytical ultracentrifugation were as in *a-f*. *a*, Southwold  $\delta$  (no B); *b*, Mildenhall  $\delta$  (no B); *c*, Mildenhall  $\delta$  (one B); *d*, Mildenhall  $\delta$  (two B); *e*, Foxhole  $\delta$  (no B); *f*, Foxhole  $\delta$  (one B); *g*, Mildenhall  $\delta$  (bulk extract) (no B); *h*, Mildenhall  $\delta$  (bulk extract) (one B).

aturation conditions and equilibrium sedimentation patterns in neutral CsCl and actinomycin D–CsCl gradients. Within the limits of resolution of these experiments, it has been shown that the B chromosomes contain representatives of the spectrum of repeated sequence families present on the A chromosomes: there are no discrete B chromosome fractions of DNA which form satellites, or which are not homologous to A chromosome DNA when they are hybridised together.

Although these studies demonstrate that the presence of distinct satellite DNA is not a general property of B chromosomes *per se*, the *Myrmeleotettix* satellite could well reflect the origin and composition of that particular B chromosome system. The large, varying amounts of satellite demonstrated in this grasshopper, however, were far greater than expected from the measured size of the B chromosome. The unusually large variations in main-band and satellite buoyant densities from different populations are also difficult to explain. As part of a general study of interpopulation differences of satellite DNA in Orthoptera and *Drosophila*, the anomalous situation in this grasshopper has been re-examined.

Male and female adult individuals of *Myrmeleotettix maculatus* were collected during August and September 1975 from wild East Anglian populations in the Mildenhall/Foxhole Warren area, where a 50% B chromosome frequency is found. Others were collected from a coastal population near Southwold, where no B chromosomes occur. Testes and ovaries were vivisected in cold isotonic saline and cleaned of fat body. Part of each testis or ovary was placed in acetic alcohol fixative before determination of the B chromosome status in propionic orcein squash preparations. The remainder was used fresh for DNA extraction or stored in a deep freeze until required. Females were injected with colcemid 1 h before dissection, to facilitate the analysis of ovariole mitoses.

DNA extracts were obtained both from single individuals and from the bulk extraction of the homogenised gonads of 25 individuals with, and 25 without, B chromosomes. Details of the two methods are given in the legend to Fig. 1. The methods of extraction were identical to those already published<sup>7,8</sup>, to ensure direct comparability of results. Pronase-treated lysates of individual gonads were loaded directly into analytical cells of an MSE Centriscan centrifuge, in neutral CsCl. The DNA of bulked gonads, after removal of protein, polysaccharide and RNA contaminants, was sedimented to equilibrium in neutral CsCl and actinomycin D–CsCl gradients. All gradients were scanned with Schlieren visible light optics to detect the presence of glycogen and other ultraviolet (254 nm) and visible light (540 nm) absorbing contaminants. If present, such extraneous ultraviolet-absorbing peaks have been removed from the traces for clarity.

A selected range of ultraviolet absorbance profiles of grasshopper DNA, after sedimentation to equilibrium in neutral CsCl, are shown in Fig. 1. No satellites to the main band were found in any of the grasshoppers with 0, 1 or 2 B chromosomes; of either sex, in any population sampled. The main-band buoyant densities and the overall mean buoyant density and standard error are given in Table 1. No significant differences were observed between genotypes or between populations.

Profiles of grasshopper DNA after sedimentation to equilibrium in actinomycin D–CsCl gradients showed a skewed spread of main-band DNA, indicating considerable heterogeneity in base composition (Fig. 2), causing differential binding of the antibiotic to fractions of varying GC content. No main-band cryptic satellites nor changes in main-band skew or spread were revealed in such gradients in the presence of B-chromosome DNA. It is interesting that direct loading and analytical centrifugation of comparable lysates of individual testes,

Table 1 Buoyant densities of main-band DNA

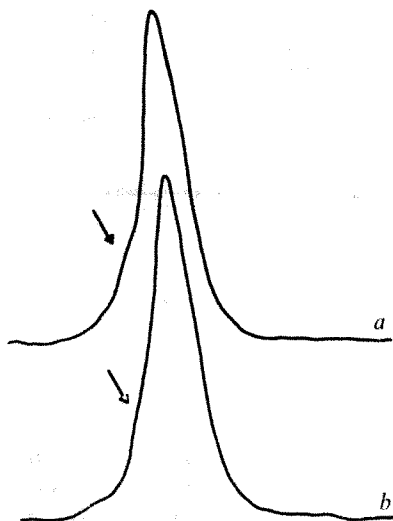
Population	Sex	B-chromosome constitution	Main-band buoyant density (g cm <sup>-3</sup> )
<i>Single individuals, direct lysates</i>			
Southwold	♂	0	1.7004
Southwold	♂	0	1.7000
Mildenhall	♂	0	1.7010
Mildenhall	♂	1	1.7009
Mildenhall	♂	1	1.7001
Mildenhall	♂	2	1.7004
Mildenhall	♂	2	1.7002
Mildenhall	♂	2	1.7003
Foxhole Warren	♂	0	1.6998
Foxhole Warren	♂	0	1.7007
Foxhole Warren	♂	0	1.7002
Foxhole Warren	♂	1	1.7003
Foxhole Warren	♂	1	1.7004
Foxhole Warren	♂	1	1.7004
Foxhole Warren	♂	1	1.7001
Foxhole Warren	♂	1	1.7002
<i>Bulk extract from 25 individuals</i>			
Mildenhall	♂	0	1.6986
Mildenhall	♂	1	1.6986
Mean = 1.7001 ± 0.00014			

Buoyant densities of main-band DNA of testes or ovaries of single individuals or bulked individuals with or without B chromosomes, were calculated<sup>12</sup> relative to the marker *Pseudomonas aeruginosa* (density = 1.726 g cm<sup>-3</sup>). Mean buoyant density and standard error are given.

ovaries and embryos of *Locusta migratoria* and *Schistocerca gregaria* in both neutral CsCl and actinomycin D-CsCl gradients have revealed the presence of previously undetected<sup>8</sup> GC-rich satellites. These satellites have been isolated and further characterised (in preparation).

The remarkable consistency of main-band densities between different individuals and different populations (Table 1), allows for some confidence to be placed in the procedures used. The lack of a resolvable satellite (Fig. 1) in the presence of B chromosomes in both males and

Fig. 2 Analytical ultraviolet absorbance patterns in actinomycin D-CsCl gradients of DNA of bulked testes with or without B chromosomes, after sedimentation to equilibrium for 20 h at 44,000 r.p.m. at 25 °C. DNA and actinomycin D were dissolved in a ratio of 1:2 in 0.5 ml of 10 mM Tris-HCl, 1 mM EDTA, pH 6.8 and brought to an initial density of 1.660 g cm<sup>-3</sup> with solid CsCl (ultracentrifuge grade). a, From bulked testes of 25 individuals without B chromosomes; b, from bulked testes of 25 individuals with one B chromosome. Note absence of cryptic main-band satellites and increased spread and skew of main-band DNA.



females throws some doubt on the reported B chromosome-satellite DNA correlation in this species. The present results suggest considerable similarity in base composition of A and B chromosome DNA, as found in several plant species<sup>9-11</sup>.

It is difficult to explain the presence of satellite DNA and the variation in satellite and main-band base composition previously reported. An exclusion of B-chromosome responsibility for the presence of the satellite leaves the possibilities of either an unrelated form of intra-population variation for satellite, or a pathogen or glyco-gen contamination.

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## Mechanism of differential Giemsa method for sister chromatids

THE 33258 Hoechst fluorescence<sup>1</sup> and 33258 Hoechst-Giemsa<sup>2</sup> methods for differential staining of bromodeoxyuridine (BrdU)-incorporated chromatids are attractive because of their rapidity and high sensitivity in revealing small sister chromatid exchanges which escape detection by autoradiography. Since carcinogen-induced chromatid breaks are associated with sister chromatid exchanges<sup>3</sup> and breakage formation parallels carcinogenesis in typical cases<sup>4,5</sup>, these methods are essential for understanding the role of chromosome aberrations in carcinogenesis. We show here that other photosensitive dyes such as thionin can replace 33258 Hoechst in the latter method and that the mechanisms of the differential Giemsa method involves photolysis of the BrdU-substituted DNA combined with these photosensitive dyes.

Fresh bone marrow cells from Long-Evans rats were cultured for two rounds of cell cycle or 26 h in thymidine-free F12 medium containing 10% foetal calf serum and 2 µg ml<sup>-1</sup> BrdU. Colchicine was added 2 h before collection. Cells were collected, treated with 0.075 M KCl, fixed in 1:3 acetic methanol, spread and air dried on glass slides. Our basic procedure consisted of 33258 Hoechst treatment, exposure to light and Giemsa staining. In addition to 33258 Hoechst, various metachromatic dyes such as thionin, toluidine blue, methylene blue, and neutral red were used successfully. They were dissolved in distilled water at a concentration of 10<sup>-3</sup>-10<sup>-8</sup> M. Chromosome specimens were dipped in distilled water, treated with the above dye solutions for 10 min, rinsed in water, mounted with 0.16 M sodium phosphate and 0.04 M sodium citrate (pH 7.0)<sup>1</sup>, and exposed to strong sunlight (noon, clear summer days) for 5 min-3 h. Thereafter, the coverslips were removed and



the cells were stained for 15 min in 2% Giemsa (Merck) solution in 0.05 M phosphate buffer (pH 6.8). Table 1 indicates that differential chromatid staining depends on the amount of 33258 Hoechst bound and the exposure to light. For routine staining,  $10^{-4}$  M 33258 Hoechst (5 mg per 100 ml) with exposure to sunlight for 15–30 min or  $10^{-3}$  M

**Table 1** Influence of 33258 Hoechst and period of exposure to sunlight on differential Giemsa staining of sister chromatids

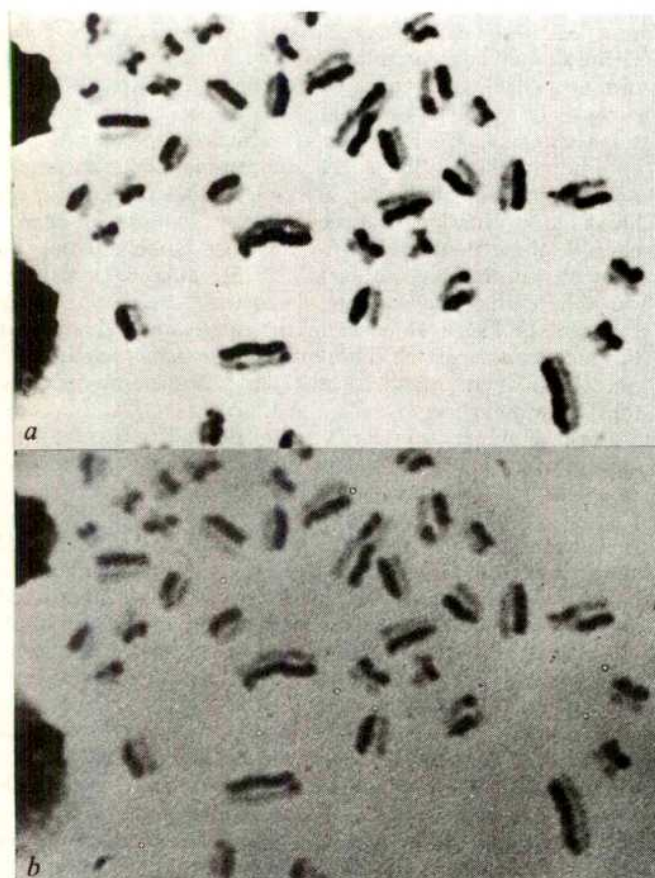
Concentration of 33258 Hoechst (M)	Period (min) of exposure to sunlight						
	5	10	15	30	60	120	180
$10^{-3}$	++	++	++	++	++	++	++
$10^{-4}$	++	++	++	++	++	++	++
$10^{-5}$	++	++	++	++	++	++	++
$10^{-6}$	—	—	—	+	++	++	++
$10^{-7}$	—	—	—	—	+	++	++
$10^{-8}$	—	—	—	—	—	±	+

++, Excellent differentiation; +, moderate; ±, trace; —, negative

thionin (23 mg per 100 ml) with exposure time of 2 h gave an excellent differential staining (Fig. 1a). A longer exposure time was necessary with other dyes. Heating in  $2\times$ SSC or in water at  $60^{\circ}\text{C}$  for 2 h (ref. 2) was not essential because differential staining was obtained in the specimens maintained at  $20\pm 2^{\circ}\text{C}$  during the whole course of the staining procedure.

Latt suggested that the fluorescence of 33258 Hoechst

**Fig. 1** Metaphase bone marrow cell grown for 26 h in BrdU-containing medium. ( $\times 3,300$ ). a, Sister chromatids differentially stained by Giemsa after treatment with  $10^{-3}$  M thionin and 2 h exposure to sunlight. b, Same cell showing differential Feulgen reaction.



bound to the BrdU-substituted chromatids was quenched because the fluorescence efficiency of this dye bound to poly(dA-BrdU) was 20 per cent of that bound to poly(dA-dT)<sup>1</sup>. Ikushima and Wolff<sup>2</sup> and Perry and Wolff<sup>3</sup> considered, however, that this hypothesis is unable to explain their 33258 Hoechst-Giemsa method and supposed that the differential Giemsa staining is caused by binding of proteins. To test this possibility, chromosome specimens differentially stained with the above 33258 Hoechst-light-Giemsa method, after destaining in 0.01 M HCl, were treated with 0.01% trypsin in saline at the room temperature for 10–20 s (ref. 7) and restained with 2% Giemsa. This caused G-banding of the darkly Giemsa-stained chromatids but failed to re-establish the affinity of the dully stained chromatids to Giemsa dye. Therefore, proteins do not seem to be involved in the differential staining. On the other hand, Feulgen reaction<sup>4</sup> carried out on the specimens treated with 33258 Hoechst or thionin and exposed to light gave differential staining (Fig. 1b). This strongly suggests that BrdU-substituted DNA undergoes rapid photolysis in the presence of photosensitive dyes and strong light.

The exact mechanism of the photolysis is unknown. BrdU-substituted DNA, however, is susceptible to light and the BrdU-substituted DNA exposed to light and treated with alkali leads to breaks<sup>5</sup>. On the other hand, the fluorescence of 33258 Hoechst and acridine orange<sup>10</sup> is quenched in the presence of halogenated base analogues possibly by strong magnetic field of the halogen atoms<sup>11</sup>. Therefore, it is possible that the light-activated dyes situated near the base analogues may act as an alkali causing chain breaks and disorganisation of the chromatid DNA. Further studies with purified BrdU-substituted DNA is in progress.

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## Thermolabile transfecting DNA from temperature-sensitive mutant of phage $\phi 29$

THE chromosome of bacteriophage  $\phi 29$ , one of the smallest *Bacillus* phages, is a non-permuted linear duplex DNA molecule of molecular weight  $11 \times 10^6$  (refs 1 and 2) that seems to be circularised by association with a protein, as circular monomers are converted into linear monomers by treatment with proteolytic enzymes<sup>3</sup>. A similar circular DNA-protein complex has been reported for adenoviruses<sup>4</sup>.

DNA extracted from phage  $\phi 29$  is infectious (transfectious) when added to competent cells of *B. subtilis*<sup>5</sup>. This transfectivity is sensitive to proteolytic enzymes<sup>6</sup>, indicating that some protein is associated with  $\phi 29$  DNA and is required for transfection. Protease-sensitive transfection has also been observed with DNAs of *B. subtilis* phages, GA1 (ref. 7), SPO2 and  $\phi 105$  (H. Hirokawa, personal communication).

The biological function of the  $\phi 29$  protein required for transfection is not known. It has been shown<sup>8</sup> that genetic markers on half- and quarter-length  $\phi 29$  DNA fragments can be rescued using competent cells super-infected with mutant phage, suggesting that DNA-bound protein is not essential for DNA uptake. It has also been shown that protease-treated GA1 phage DNA competes with transforming DNA to the same extent as untreated GA1 DNA<sup>7</sup>. Apparently the protein associated with phage DNA is needed at some stage of transfection other than uptake. Since a specific DNA-bound protein is required for transfection, one might expect to find transfection-defective mutants with altered DNA-protein complexes. Such mutants could aid in defining the role of the protein in DNA transfection. We show here that a temperature-sensitive DNA synthesis mutant of  $\phi 29$  originally isolated by Talavera *et al.*<sup>9,10</sup> produces thermolabile transfecting DNA.

Initially, we anticipated that mutant phages containing a thermolabile DNA-protein complex might be temperature-sensitive for viability. We therefore searched for a  $\phi 29$  mutant the viability of which was temperature-sensitive. One of the temperature-sensitive DNA synthesis mutants, *tsK132* (provided by Dr E. Viñuela) was found to be significantly more heat-sensitive than wild type (Fig. 1). Subsequently, we demonstrated that in transfection experiments *tsK132* DNA is also highly thermolabile. Kinetics of the loss of transfectivity as a function of time of heating at 65 °C are shown in Fig. 2. Since transfectivity of DNA samples was determined at 30 °C, the thermosensitivity of *tsK132* DNA is irreversible. Loss of transfectivity at a temperature lower than the melting temperature of  $\phi 29$

Fig. 1 Comparison of the heat stability of wild-type  $\phi 29$  (○) and *tsK132* phage particle (●). Phages ( $1 \times 10^8$  PFU ml<sup>-1</sup>) in phage adsorption medium (0.1 M NaCl,  $10^{-2}$  M MgSO<sub>4</sub> in 0.1 M Tris-HCl buffer, pH 7.0) were heated at 70 °C for the indicated times. After dilution, phages were titred at 30 °C using *B. amyloliquefaciens* H as indicator.

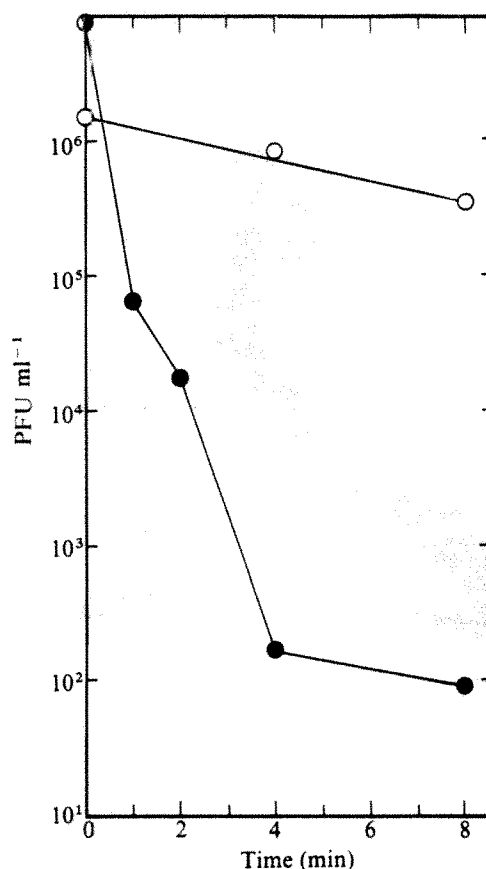
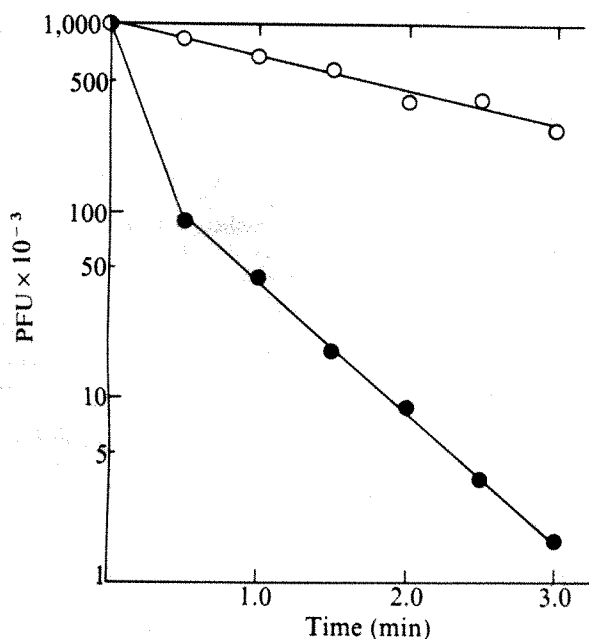


Fig. 2 Comparison of thermostability of transfectious DNA from wild-type  $\phi 29$  (○) and *tsK132* (●). Purified phage particles were disrupted by treatment with 1% Sarkosyl for 3 min at 40 °C. Transfectious DNA was phenol extracted and dialysed extensively against standard saline citrate in 0.01 M Tris-HCl buffer, pH 7.0, containing 0.1 mM EDTA. Each DNA solution (0.5  $\mu$ g ml<sup>-1</sup>) was heated for the indicated times and used for transfection. Transfectivity was determined at 30 °C using competent cells of *B. subtilis* 168 according to the method of Reilly and Spizizen<sup>5</sup>.

DNA ( $T_m$  86 °C)<sup>11</sup> suggests that this thermosensitivity results from an alteration in the protein associated with the DNA. The *K* gene of  $\phi 29$  is one of four genes acting on DNA synthesis early in infection<sup>12</sup>. Therefore, it was of considerable interest to establish that the same mutation affects both DNA replication and transfection.

DNA synthesis experiments were carried out to ascertain whether a purified reisolate of *tsK132* was temperature-sensitive for DNA replication. Thymine-requiring cells infected with phages were incubated in a medium containing tritiated thymidine and 6-(*p*-hydroxyphenylazo)-uracil (HPUra), added to inhibit host DNA synthesis<sup>9,13</sup>. Aliquots were removed at different times for determination of radioactivity in acid-insoluble material. Phage DNA synthesis in *tsK132*-infected cells is almost completely inhibited at 45 °C, whereas it is normal at 30 °C. A shift-up experiment was carried out to determine whether the temperature-sensitive function determined by the *K* gene is required continuously for DNA synthesis or whether it is completed at a definite time during the latent period. Cultures infected and incubated at 30 °C were shifted to 45 °C at different times and DNA synthesis was measured (Fig. 3a). Phage DNA synthesis in cells infected with the mutant stopped immediately when the cells were shifted from 30 to 45 °C. Therefore, the functional product of the *K* gene is required continuously for  $\phi 29$  DNA synthesis, and in mutant *tsK132* this product is inactivated immediately at 45 °C.

The results of shift-down experiments (Fig. 3b) indicate



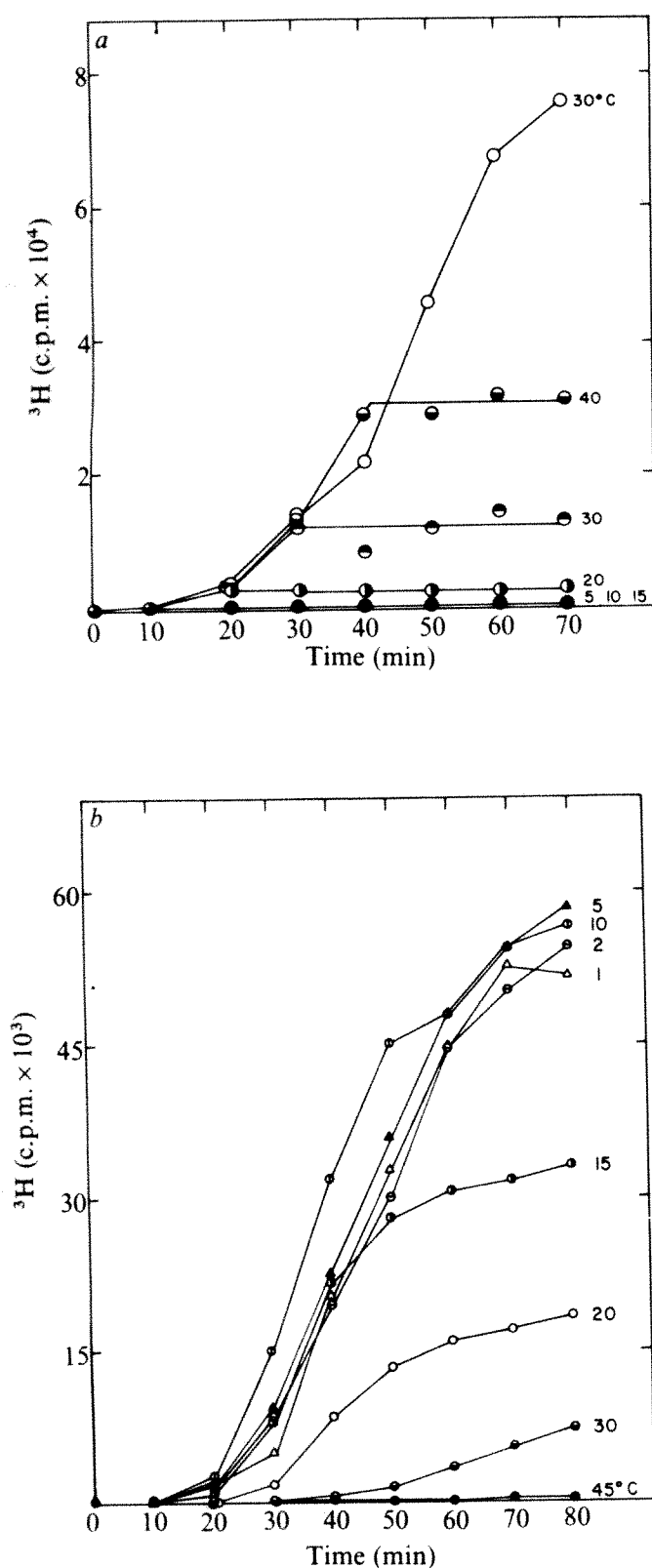


Fig. 3 Effects of temperature shift-up and shift-down on DNA synthesis in *tsK132*-infected cells. *B. subtilis* SCR 115 (*spoA12 thyA, B*) was grown to  $2 \times 10^8$  cells  $\text{ml}^{-1}$  in Penassay broth containing  $2 \mu\text{g ml}^{-1}$  thymine at 37°C, centrifuged, and resuspended in adsorption medium. Cells were mixed with phages at a multiplicity of 20 and incubated for 10 min at room temperature to enable adsorption. Bacteria-phage complexes were then transferred into  $^3\text{H}$ -thymidine incorporation medium consisting of Penassay broth,  $50 \mu\text{g/ml}$  HPURa and  $10 \mu\text{Ci/ml}$   $^3\text{H}$ -thymidine. Cultures were incubated with shaking at either 30°C(a) or 45°C(b). Infected cultures were shifted to the other temperature at the indicated times (min). Samples were removed, treated with 10% cold TCA, filtered, washed with 5% TCA-containing  $100 \mu\text{g ml}^{-1}$  thymidine and counted.

that shifts carried out up to 10 min after infection do not affect the ability of cells infected with *tsK132* to synthesise phage DNA. After this time, shift-down results in depressed levels of DNA synthesis. These results agree with those of Talavera *et al.*<sup>9,10</sup> in showing that mutant *tsK132* is deficient in DNA replication *in vivo*. Since both RNA and protein synthesis are required for  $\phi 29$  replication<sup>14</sup>, we examined the possibility that phage RNA synthesis is also shut off immediately after shifting the temperature from 30 to 45°C. RNA was extracted from *tsK132*-infected cells at various times after shifting from 30 to 45°C and hybridised to denatured  $\phi 29$  DNA immobilised on nitrocellulose filters. The results (not shown) indicate that phage messenger RNA (mRNA) synthesis is not blocked immediately after shifting from 30 to 45°C. Chloramphenicol added before the onset of DNA synthesis inhibits  $\phi 29$  DNA replication. It does not, however, shut off DNA synthesis immediately when added after 10 min of infection (not shown). These findings suggest that the immediate cessation of DNA synthesis in *tsK132*-infected cells at non-permissive temperatures is not secondary to an inability to synthesise phage-specific RNA or protein. Rather, it seems more likely that an essential component of the DNA-synthesising machinery is itself thermosensitive.

Reversion studies indicate that the *tsK132* mutant is a point mutation. In four isolated plaques of *tsK132* grown at 30°C, revertants appeared at an average frequency of  $5 \times 10^{-6}$ . Experiments with three *tsK132* revertants showed that they were indistinguishable from the wild type at 30 and 45°C in growth and thermostability of transfecting DNA. One of the revertants was crossed with the wild-type phage and 1,450 progeny phages were tested for temperature sensitivity. No *tsK132* segregants were found. We conclude that *tsK132* is a single-base change mutant and that reversion can occur at or near the site of the original lesion. Our results, therefore, together with those of Talavera *et al.*<sup>9,10</sup>, show that a single mutation affects both DNA replication and transfection. Consequently, it seems that at least one DNA-associated protein contained in the phage particle is involved in  $\phi 29$  DNA transfection. We have not yet identified the protein.

The possibility that the *K* protein is involved in circularisation of  $\phi 29$  DNA and that this is essential for transfection, seems remote since the circular protein-DNA complex of phage  $\phi 29$  can be disrupted by treatment with sodium dodecyl sulphate<sup>3</sup>. Such DNA is active in transfection (F. K. and J. I., unpublished). Another possible explanation for our results would be that the DNA-protein complex is resistant to attack by nucleases present in competent cells. Although this possibility cannot be ruled out, it also seems unlikely since protease-treated  $\phi 29$  DNA is able to rescue most of the genetic markers of  $\phi 29$  mutants (F. K. and J. I., unpublished).

Instead, in analogy with the *coli* phage M13 system<sup>15,16</sup>, it seems more likely that the  $\phi 29$  *K* gene specifies a 'pilot' protein essential for  $\phi 29$  DNA replication. The very same protein or a fragment thereof could be incorporated into the  $\phi 29$  phage particle during phage maturation as an integral protein which is required for  $\phi 29$  DNA transfection. Note that Ivarie and Pène<sup>14</sup> found that after infection parental  $\phi 29$  DNA is associated with host cell membrane. They described two mutants, one temperature-sensitive and the other suppressor-sensitive; both are unable to form DNA-membrane complexes and are defective in DNA replication<sup>17</sup>. Although the nature of the *K* gene protein and its function(s) are not known, further studies of this protein may clarify the role of DNA-associated proteins in DNA replication.

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## Involvement of DNA in resistance of potatoes to invasion by *Phytophthora infestans*

THE primary event that determines whether or not a plant becomes diseased is a mutual recognition which brings the plant into contact with the pathogen. Flor<sup>1</sup> developed the gene-for-gene theory from his studies of the relationship between flax and rust. This theory implies that the genes in the plant that condition the reaction can be identified only by their interaction with specific races of the pathogen, and the genes in the pathogen that condition pathogenicity can be identified only by their interaction with a specific cultivar of the plant. Allen<sup>2</sup> reported that nucleic acid or protein was involved in the resistance of the plant to the pathogen. Ledoux *et al.*<sup>3</sup> found that labelled DNA from bacteria could be incorporated into barley roots and become combined with the DNA in the nuclei of the plant cells. Thus DNA may have a role in the recognition of the plant by the pathogen. We have obtained support for this notion. We painted potato midribs and tubers with DNA fractions<sup>4</sup> from different potato cultivars and species of Solanaceae, and found that their susceptibility to *Phytophthora infestans* was affected.

When petiole midribs of potato cultivar Norin No. 1 (gene *r*, for susceptibility) were inoculated with zoospores of *P. infestans* (race 0), only lesions were observed, but when the DNA fraction of the resistant interspecific hybrid 96-56 (gene *R*<sub>1</sub>, for resistance) was applied to midribs of Norin No. 1, both lesions and hypersensitive flecks were found, and the cells invaded by the pathogen soon died (Fig. 1). Hypersensitive flecks are signs of premature dying off (necrosis) of the infected tissue as well as inactivation and localisation of the infectious agent, and are associated with the attack of a resistant plant by

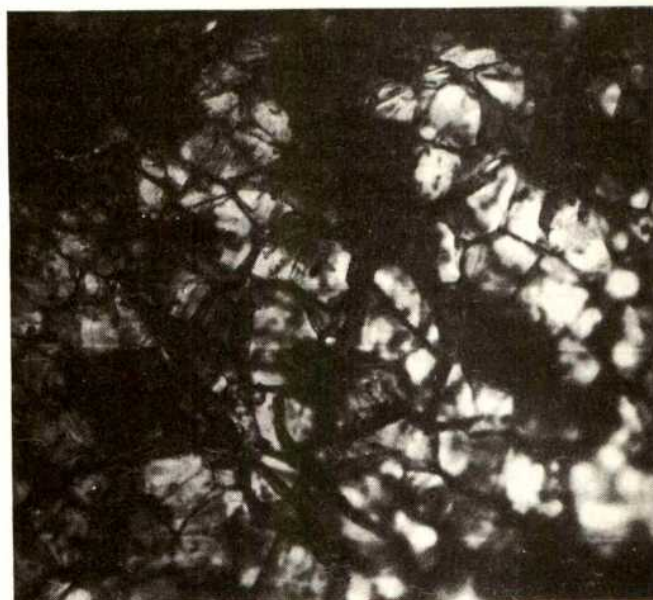


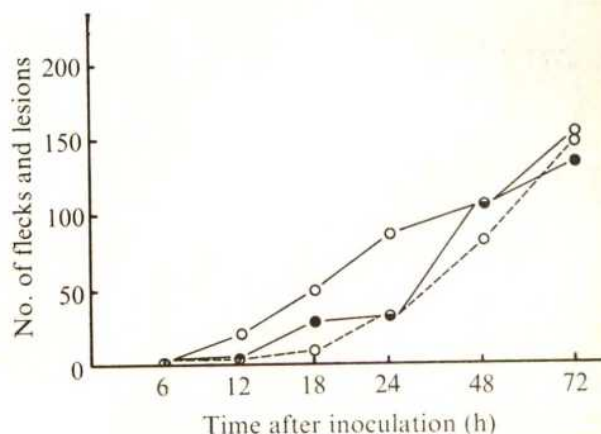
Fig. 1 Lesions and hypersensitive flecks of late blight on potato tubers of Norin No. 1 painted with the DNA fraction of the hybrid. The DNA fraction was prepared by Thomas' method<sup>4</sup>. The concentration of DNA in the water solution was 455  $\mu\text{g ml}^{-1}$ . Painting was done with a sterilised brush. *P. infestans* was inoculated 1 h after painting. ●—●, Lesions (control); ○—○, flecks (painted tuber); ○—○—○, lesions (painted tuber).

incompatible races of *P. infestans*; lesions result from the invasion of a plant by compatible races of the pathogen.

Results were similar with tubers of hybrid 96-56 in contact with the DNA fraction of Norin No. 1, but the control (Norin No. 1, not painted with the DNA fraction of hybrid 96-56) had only lesions. On the tubers of hybrid 96-56 treated with the DNA fraction from Norin No. 1, both mycelia and conidia developed, similar to those observed in the control. The conidia on the hybrid tuber were  $25.9 \times 17.7 \mu\text{m}$ , somewhat smaller than those of the control ( $27.7 \times 18.1 \mu\text{m}$ ) (figures are the average of 50 conidia) (Fig. 2).

When petiole midribs of potato hybrid 96-56 were treated with the DNA fraction of tomato (cultivar Fukuju No. 2) both lesions and hypersensitive flecks were observed

Fig. 2 Mycelial growth of *P. infestans* on hybrid tuber painted with the DNA fraction of Norin No. 1. The concentration of DNA in the water solution was 457  $\mu\text{g ml}^{-1}$ . *P. infestans* was inoculated 1 h after painting.





**Table 1** Lesions and hypersensitive flecks of late blight on potato interspecific hybrid after treating with DNA fraction from tomato

	No. of flecks and lesions observed	No. of hypersensitive flecks	%	No. of lesions	%
DNA from tomato* on hybrid†	396‡	221	35.8	175	44.2
Hybrid (control)	229	229	100.0	0	0.0

\*The concentration of DNA in the water solution was 433 µg ml<sup>-1</sup>.

†Interspecific hybrid 96-56 (*R<sub>1</sub>* gene).

‡72 h after inoculation of *Phytophthora infestans* (race 0).

(Table 1). Similar lesions and flecks developed when the petiole of Norin No. 1 was treated with the DNA fraction of jimson weed (*Datura abla* Nees) and tobacco (*Nicotiana tabacum* L. cultivar xanthi nc), but in the control (Norin No. 1 without painting the DNA of jimson weed and tobacco) only lesions were found (Table 2).

Electrophoresis revealed an increase in the amount of protein in the leaves of the susceptible cultivar after treatment with the DNA fraction from resistant hybrid, before it was inoculated with pathogen. A new protein band, coinciding in *R<sub>F</sub>* with a protein in the resistant hybrid, appeared 6 h after inoculation of an incompatible race of *P. infestans*. Potato leaves painted with the DNA fraction of different cultivars had more peroxidase and phenylalanine ammonia lyase activity than the control (without painting DNA).

**Table 2** Lesions and hypersensitive flecks of late blight on potato painted with the DNA fraction from jimson weed and tobacco

	No. of flecks and lesions observed	No. of hypersensitive flecks	%	No. of lesions	%
DNA from jimson weed* on Norin No. 1	896*	309	34.5	587	65.5
Norin No. 1 (control)	781	0	0.0	781	100.0
DNA from Tobacco† on Norin No. 1	1,093	237	21.7	865	78.3
Norin No. 1 (control)	699	0	0.0	699	100.0

\*The concentration of DNA in the water solution was 135 µg ml<sup>-1</sup>.

†The concentration of DNA in the water solution was 300 µg ml<sup>-1</sup>.

‡72 h after inoculation of *Phytophthora infestans* (race 0).

A large quantity of rishitin has been reported on the tubers of susceptible cultivars treated with the DNA fraction from the resistant hybrid<sup>6</sup>. Rishitin<sup>8,9</sup> is one of several phytoalexins, which were believed to be important for the hypersensitivity<sup>7</sup>. Rishitinol<sup>9</sup>, lubimin<sup>10</sup> and phytuberin<sup>11</sup> were also identified as phytoalexins in potatoes infected with *P. infestans*. Király *et al.*<sup>12</sup>, however, showed that this hypersensitive necrosis was a consequence, not the cause of resistance of potato, beans and wheat to various fungi. We have described<sup>13-16</sup>, however, the role of DNA fractions of potatoes in the creation of hypersensitivity to invasion by *P. infestans*. These and our results support the idea that DNA is directly involved in the specificity of fungal pathogen for the plants.

Details of this work will be published elsewhere. We

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## New class of enzymes acting on damaged DNA

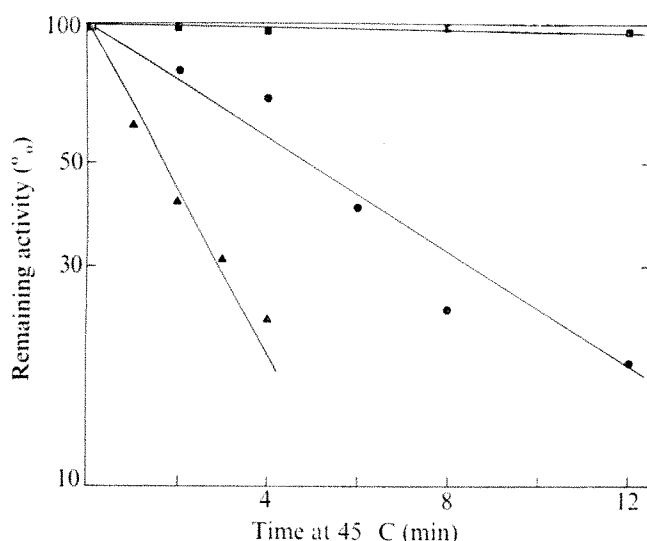
ONE of the major pathways of DNA repair consists of the removal and replacement of damaged nucleotides in non-replicating DNA. In the classical excision repair model<sup>1,2</sup>, the first enzymatic step in this process is the introduction of a single-strand break in the DNA adjacent to a defective nucleotide residue. Endonucleases that specifically attack DNA containing pyrimidine dimers<sup>2</sup> or apurinic sites<sup>3-5</sup> have subsequently been isolated from many types of cells, and enzymes of these two types have been shown to be active in DNA repair in *Escherichia coli* by the isolation of repair-deficient mutant strains with defective endonucleases<sup>6,7</sup>.

An enzyme that specifically attacks DNA containing deaminated deoxycytidine monophosphate residues has also been detected in *E. coli*<sup>8</sup>. This enzyme is not, however, a regular nuclease, because it catalyses the hydrolysis of the N-glycosidic bonds of the damaged nucleotide residues, but it does not cleave any phosphodiester bonds. The reaction products after the action of this N-glycosidase on deaminated DNA are consequently free uracil and partly depyrimidinated DNA of unaltered chain length. Similar types of enzyme activities that release free 3-methyladenine (3-MeAde) and 6-methoxyguanine from alkylated DNA were discovered by Kirtikar and Goldthwait in a purified but non-homogeneous preparation of *E. coli* endonuclease II, and were ascribed to the endonuclease<sup>9</sup>. As the different DNA N-glycosidase activities may act together with endonuclease II in DNA repair, it seemed of interest to define the relationship of these enzyme activities to each other. In the present work, it is shown that the *E. coli* Ura-DNA glycosidase, 3-MeAde-DNA glycosidase, and endonuclease II activities are attributable to three different enzymes.

The three enzyme activities studied showed similar fractionation properties on streptomycin treatment, ammonium



sulphate fractionation, and gel filtration on Sephadex G-100 of an *E. coli* cell extract. On incubation at 45 °C of an enzyme fraction purified by these steps, however, the three activities were inactivated at different rates (Fig. 1). Endo-



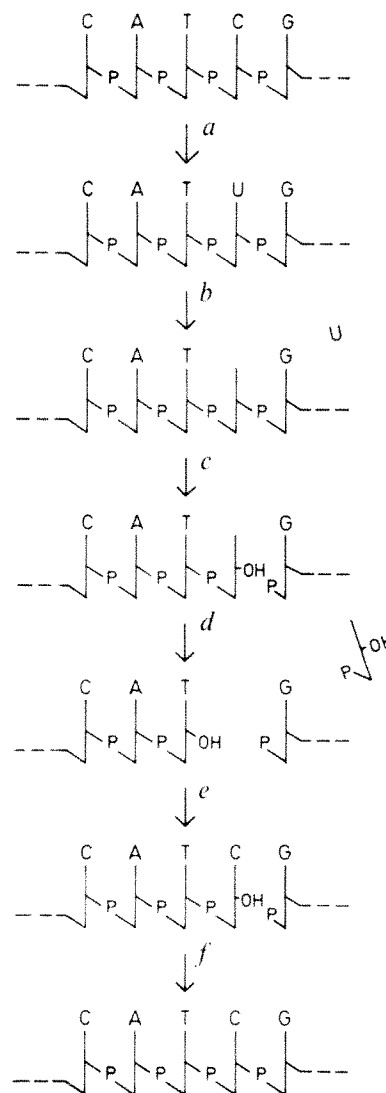
**Fig. 1** Rates of heat inactivation at 45 °C of *E. coli* Ura-DNA glycosidase (■), 3-MeAde-DNA glycosidase (●) and endonuclease II (▲) activities. Activities were copurified from a crude cell extract of *E. coli* 1100 (endo I<sup>-</sup>) by streptomycin treatment, ammonium sulphate fractionation and gel filtration on Sephadex G-100 (ref. 8). Active protein fraction was dialysed against 0.2 M NaCl-0.05 M potassium phosphate (pH 7.4) and 0.001 M dithiothreitol, and aliquots were heated at 45 °C for indicated times before assay. Ura-DNA glycosidase was assayed by measuring the release of free uracil from partly deaminated DNA, initially radioactively labelled in the cytosine residues<sup>8</sup>. One enzyme unit releases 1 nmol uracil from DNA in standard reaction conditions. Endonuclease II was assayed by a modification of the method of Verly *et al.*<sup>4,16</sup>. One enzyme unit releases 1 nmol <sup>32</sup>P in acid-soluble form from the radioactive, partly depurinated DNA substrate in standard reaction conditions. 3-MeAde-DNA glycosidase was assayed by measuring the release of free 3-MeAde from alkylated DNA (ref. 9), as follows. *B. subtilis* DNA, <sup>14</sup>C-labelled in purine residues, was prepared as described previously<sup>17</sup>. DNA (0.3 mg ml<sup>-1</sup>, 4 × 10<sup>7</sup> c.p.m. mg<sup>-1</sup>) was incubated with 0.25 M methyl methanesulphonate in 0.1 M Tris-HCl and 10<sup>-3</sup> M trisodium citrate (pH 8.0) at 37 °C for 30 min. One-tenth volume 5 M NaCl and two volumes ethanol (chilled to -20 °C) were added at 0 °C, and the DNA fibres collected, washed in 80% ethanol, dissolved in 1 M NaCl, 0.01 M Tris-HCl and 0.001 M EDTA (pH 7.8), and dialysed for 16 h against the same buffer, followed by 3 h against buffer without NaCl. Alkylated DNA was stored in several small aliquots at -70 °C. This DNA contained 0.9% of the total radioactivity as 3-MeAde, as determined by paper chromatographic analysis of an acid hydrolysate in methanol-concentrated HCl-H<sub>2</sub>O (7:2:1, ref. 18). 3-MeAde was quantitatively released from DNA by incubation at 100 °C for 10 min at neutral pH (ref. 18). In enzyme assays, the reaction mixture (25 µl) contained 0.07 M HEPES-KOH (pH 7.8), 10<sup>-3</sup> M dithiothreitol, 2 µg alkylated <sup>14</sup>C-DNA and an amount of enzyme sufficient to cause 10-50% release of 3-MeAde. After 20 min incubation at 37 °C, mixtures were chilled to 0 °C and individually supplemented with 5 µl 0.5% 3-MeAde (Cyclo Chemicals), 3 µl 5M NaCl, 10 µl 0.2% heat-denatured calf thymus DNA and 90 µl ethanol (chilled to -20 °C). After 1 h at -10 °C, samples were centrifuged at 2 °C, and 100 µl supernatant were recovered. This material, which contained <3% initial radioactivity, was applied to a Whatman 3MM paper at room temperature (21 °C) and analysed by descending paper chromatography for 17 h in a system containing a saturated ammonium sulphate solution, 0.1 M phosphate (pH 7.2) and isopropanol (79:19:2, ref. 19). After drying, 3-MeAde spot was localised by its ultraviolet absorbance, cut into pieces and transferred to a scintillation vial, and eluted with 5 ml H<sub>2</sub>O for at least 1 h. Fifteen millilitres Aquasol (New England Nuclear) were then added, and the radioactivity was determined in a liquid scintillation spectrometer at a counting efficiency of 80%. One enzyme unit releases 1 nmol 3-MeAde in conditions described. In the absence of enzyme, 3% total 3-MeAde in the DNA was released by hydrolysis<sup>18</sup> during incubation. The identity of the enzymatically released material with authentic 3-MeAde was confirmed in three additional chromatography systems.

nuclease II activity was most heat-labile, with 50% of the activity remaining after about 100 s. 3-MeAde-DNA glycosidase activity was inactivated at a rate three times slower and had 50% residual activity after 5 min. Ura-DNA glycosidase activity remained practically unchanged after 12 min at 45 °C, but was inactivated at 55 °C with 50% inactivation occurring after 3 min.

Direct evidence for the notion that Ura-DNA glycosidase activity depended on a separate enzyme was obtained by the physical separation of this activity from the other enzyme activities investigated by hydroxyapatite chromatography. When the Sephadex-purified enzyme fraction was applied to a hydroxyapatite column in a buffer containing 0.01 M phosphate, Ura-DNA glycosidase activity did not adsorb and was recovered in the effluent. In contrast, more than 90% of the 3-MeAde-DNA glycosidase and endonuclease II activities adsorbed to the column but were eluted with 0.3 M phosphate buffer (Table 1).

It has been shown that *E. coli* endonuclease II and exonuclease III are the same enzyme, which has been assayed by different methods<sup>7</sup>. In agreement with this notion, a preparation of exonuclease III, made according to Jovin *et al.*<sup>10</sup>, contained a high level of endonuclease II activity.

**Fig. 2** Model for repair of partly deaminated or alkylated DNA. Complementary strand in DNA double helix not shown. a, Cytosine deamination (heat or bisulphite); b, release of free uracil (N-glycosidase); c, incision (endonuclease II); d, excision (exonuclease III); e, repair replication (a DNA polymerase); f, joining (DNA ligase).



In relation to the endonuclease activity, this enzyme preparation contained four fold less 3-MeAde-DNA glycosidase and 100-fold less Ura-DNA glycosidase activities (Table 1) than the much cruder Sephadex-purified enzyme fraction used here. This indicates that 3-MeAde-DNA glycosidase activity is partly removed and Ura-DNA glycosidase largely removed during the preparation of highly purified endonuclease II/exonuclease III by the above procedure.

In view of their specificities for damaged DNA<sup>8,9</sup>, it seems likely that the DNA N-glycosidases are active in DNA repair. A hypothetical model for such repair is shown in Fig. 2. It is proposed that for some types of DNA lesions (excluding pyrimidine dimers), the specific recognition and elimination of the damaged base is due to an N-glycosidase. The apurinic (or apyrimidinic) site is then repaired by the action of endonuclease II/exonuclease III, a DNA polymerase and DNA ligase<sup>3,15</sup>. One of the advantages of this

**Table 1** Relative amounts of Ura-DNA glycosidase, 3-MeAde-DNA glycosidase, and endonuclease II in different *E. coli* enzyme preparations

Enzyme fraction	Ura-DNA glycosidase (units mg <sup>-1</sup> )	3-MeAde-DNA glycosidase (units mg <sup>-1</sup> )	Endonuclease II (units mg <sup>-1</sup> )
<i>E. coli</i> 1100, Sephadex G-100 fraction	1,050	7.0	230
Hydroxyapatite fraction A	4,100	1.5	< 5
Hydroxyapatite fraction B	50	8.0	210
<i>E. coli</i> AB1157 ( <i>xthA</i> <sup>+</sup> ), Sephadex G-100 fraction	900	6.0	260
<i>E. coli</i> NH5016 ( <i>xthA</i> <sup>-</sup> ), Sephadex G-100 fraction	1,100	6.5	25
<i>E. coli</i> exonuclease III (ref. 10)	300	50	7,000

*E. coli* Sephadex G-100 fractions were made as described<sup>8</sup> (see also Fig. 1). For hydroxyapatite chromatography, an ammonium sulphate-concentrated Sephadex fraction (23 mg protein in 6 ml) was dialysed against 0.2 M KCl, 0.01 M potassium phosphate and 10<sup>-3</sup> M dithiothreitol (pH 7.4), and applied to a column (1.3 × 11 cm) of hydroxyapatite<sup>20</sup> in the same buffer. 20% of the protein did not adsorb to the column (fraction A). The adsorbed protein was then eluted with 0.3 M potassium phosphate (pH 7.4) and 10<sup>-3</sup> M dithiothreitol (fraction B).

Several methyl methane sulphonate-sensitive *E. coli* mutants, defective in endonuclease II, have been isolated (ref. 7 and S. Ljungquist, T. Lindahl, and P. Howard-Flanders, unpublished). An enzyme preparation from one mutant of that type, *E. coli* NH5016, was investigated here together with a preparation from the parent strain, *E. coli* AB1157. As expected, the mutant had a tenfold decreased amount of endonuclease II activity. In contrast, the same mutant extract contained normal levels of both DNA N-glycosidase activities (Table 1). The above data strongly suggest that *E. coli* 3-MeAde-DNA glycosidase and endonuclease II are two different enzymes, and it is clear that neither of them is identical with the Ura-DNA glycosidase.

It has been shown with both *E. coli* endonuclease II and the similar mammalian endonuclease acting at apurinic sites that alkylated DNA is not a substrate, unless the DNA is first incubated to enable release of alkylated purines by hydrolysis<sup>4,11</sup>. Goldthwait *et al.*<sup>12</sup> have claimed that the enzyme also acts directly on alkylated DNA. The finding of 3-MeAde-DNA glycosidase activity in partly purified *E. coli* endonuclease II preparations seems to explain this discrepancy, as the concerted effect of these two enzymes on alkylated DNA would be the introduction of single-strand breaks. Similar complications have arisen in the interpretation of data on enzymes degrading uracil-containing DNA. Carrier and Setlow<sup>13</sup> showed that an apparent endonuclease activity that acted specifically on uracil-containing DNA was present in *Micrococcus luteus* extracts, but their experimental design did not separate between enzymes that introduce chain breaks and enzymes that introduce alkali-labile sites. Tomita and Takahashi<sup>14</sup> reported that *Bacillus subtilis* contains a DNase specific for uracil-containing DNA, which released deoxyuridine as a major reaction product and was inhibited after infection with phage PBS1. The chromatographic method used to identify deoxyuridine, however, does not separate this substance from free uracil. Therefore, it seems unclear at present if conventional DNases exist that specifically attack uracil-containing DNA. Note that the Ura-DNA glycosidase seems to be a widely distributed enzyme that has so far been found in extracts from *E. coli*, *M. luteus*, *B. subtilis* and mammalian cells (T.L., unpublished).

scheme over the traditional excision repair model is that DNA ligase cannot act until the damaged nucleotide residue has been removed and replaced. A more definite assessment of the physiological functions of the DNA N-glycosidases, however, will have to await the isolation of mutant cell strains with defective enzymes of this class.

I thank Dr Bill Sugden for *E. coli* exonuclease III, and Ms Barbro Nyberg for assistance. This work was supported by the Swedish Natural Science Research Council, the Swedish Cancer Society and the Karolinska Institute.

*Note added in proof:* The rapid selective degradation of uracil-containing DNA by *B. subtilis* crude cell extracts is due to a Ura-DNA glycosidase. Extracts from phage PBS 2-infected bacteria contain a factor that completely inhibits the enzyme<sup>21</sup>.

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# reviews

PTOLEMY'S (flourished AD 127–148) *Almagest* is a substantial and difficult work. Because it was the culmination of centuries of Greek astronomy, with its Babylonian background, and was the principal influence on astronomical theory up to the time of Copernicus and beyond, it has received the attention of every serious historian of astronomy. But, as Professor Pedersen says in his first chapter\*, "it has been talked about by many, but studied seriously only by the few." His aim is "to help students of the history of astronomy to understand and appreciate Ptolemy's great and classical work."

Since the mathematical and astronomical ideas of the *Almagest* are not very abstruse, the difficulties lie in the mode of presentation. Some of these difficulties were pointed out in the twelfth century by Jābir b. Aflah in his *Correction of the Almagest*. Among his many complaints, he said that the principles were obscured by numerical computations, that Ptolemy's exposition was sometimes too compressed, and that the meaning was often unclear because of the chain of translators between Ptolemy and himself. These defects Professor Pedersen has completely removed. He has elucidated the ideas behind Ptolemy's demonstrations, keeping them separate from numerical manipulations; difficult passages are examined in the light of recent scholarship; and, since he has worked from Heiberg's edition of the original Greek text, neither Professor Pedersen nor his readers have to suffer from the inaccuracies and obfuscations of translators.

Much of the clarity of this book comes from the author's ruthless use of modern notation. Not only are brackets, subscripts, square-root signs, and so on, used to describe the operations Ptolemy describes verbally, but some of his fundamental ideas are expressed in terms of functions, and some of his trigonometry is represented by the modern equivalent. Thus plane triangles are often solved in the modern way—that is, without dropping perpendiculars or circumscribing circles—and the functions *tan* and *cot*

\* *A Survey of the Almagest*. (Acta Historica Scientiarum Naturalium et Medicinalium, Volume 30.) By Olaf Pedersen. Pp. 454. (Odense University: Odense, Denmark, 1974.) Dn. kr. 200.00

## Ancient astronomical masterpiece



Ptolemy taking the altitude of a heavenly body with a quadrant (From G. Reisch, *Margarita Philosophica*, Basle, 1508)

are freely used where Ptolemy made do with chords. There is usually sufficient indication of what has been done. The result is that the reader knows that Ptolemy's exact mode of thinking has not been reported, but can see his line of reasoning without being distracted by the prolixities of archaic mathematical technique. It might be thought that modernisation is sometimes taken too far. On page 164, for instance, the mean longitude of the Moon is found by integrating its differential coefficient. While the reader may be assumed to realise that Ptolemy did not use the calculus, the modernity of the expression is startling and not really necessary. Elsewhere the anachronism is more than justified by the gain in clarity. For example, Ptolemy's calculation of lunar longitude from his general table is not easy to understand, but is fully explained in the *Survey* (pages 195–9) with the help of modern symbolism, particular attention being paid to Ptolemy's interpolative method of approximation. There are one or two mistakes in interpretation—I think, for instance, that the proof of the equivalence of the simple epicyclic and excentric models (pages 137–8) is mis-stated—

but such lapses are rare.

In some cases, Professor Pedersen goes beyond what is written in the *Almagest*, but only to make the meaning clear. Several graphs of relevant functions are included to make the astronomical model under consideration easier to understand. Further aids for the reader are to be found in Appendix B, in which all the numerical parameters calculated in the *Almagest* are collected, and in the lists of observations. Besides a general inventory of dated observations (Appendix A), the observational data for particular astronomical models are tabulated in the appropriate place in the text.

The purely historical content of the *Survey*, although not so compendious as the expository, provides a useful introduction to Alexandrian astronomy and physics. There is a short chapter on Ptolemy's other works; and his reliance, in the *Almagest* and elsewhere, on Babylonian and Hipparchan observations and parameters is discussed as they occur. The book is introduced by a chapter on the transmission of the *Almagest* up to modern times. It is completed by a large and useful bibliography.

R. P. Lorch



## Solar radiation

*Solar Gamma-, X-, and EUV Radiation.* (International Astronomical Union Symposium No. 68, Buenos Aires 1974). Organised by the IAU in cooperation with COSPAR.) Edited by Sharad E. Kane. Pp. xii + 439. (Reidel: Dordrecht and Boston, Massachusetts, 1975.) Dfl. 145; \$58.00.

DURING the past decade the rapid advance in instrumental techniques and the extensive use of orbiting observatories has provided a wealth of data on  $\gamma$ -, X-ray and EUV radiation from the Sun. The International Astronomical Union Symposium in June 1974 provided an opportunity for specialists in the field to review the observational results and to describe current theories of solar emission mechanisms and related phenomena. Highlights of the proceedings included reports of preliminary data from Skylab on the newly discovered coronal holes and bright points.

The book is split into three sections covering general solar activity, active regions and solar flares. Each section contains detailed review articles written

by leading authorities on the topics concerned, and summaries of contributed papers announcing the latest results. These include data from ground based telescopes, sounding rockets, balloons and satellites operated by American, European and Russian groups. In addition, the book contains papers on energetic particles and microwave radiation, forming a more complete picture of the solar phenomena in which they play a role. Thirty-nine papers are given in all.

The coverage of the rather broad subject suggested by the title is comprehensive, but inevitably in a collection of articles written independently by a large number of authors some duplication takes place and it is often difficult to refer to specific items of interest. The latter problem detracts a little from the value of the book as a reference volume for its intended audience of research workers and students in solar physics and solar terrestrial relations. Nevertheless, these workers will find it an important and necessary addition to the library of their respective research institutes, if a little expensive for their own bookshelves.

With the bulk of the Skylab data analysis yet to be completed and with the continued high level of activity in observational and theoretical solar studies it seems certain that this account of solar radiation will quickly be superseded. **C. G. Rappley**

but Lamarck comes in for much discussion.

I found some of the biochemical analogies in the book to be colourful, but perhaps not appropriate. For example, on page 268 the development of an egg into a vertebrate is likened to the proteolytic and lipolytic ripening of a cheese: "can develop as it were into a Cheddar, a Camembert, a Brie, etc., as well as into a Stilton".

Such an egg would become *pidan* rather than a duckling. The 'ripening' of cheese is not directed by the DNA inherently present, as in an egg. Ripening comes about by the degradative action of bacteria, as Waddington points out, by autolysis, or more usually by the growth of moulds. The "glory of ripeness" that he attributes to a Stilton cheese is the odour, taste and other properties of esters, fatty acids, and protein hydrolytic products that result from the decomposition of butterfat, lactose, casein and lactalbumin; a process that leads to putrefaction. What a contrast to the development of an egg—the fantastic spectacle of a DNA molecule at work to make another DNA molecule.

See also the description of protein structure on page 190, where the author says that all but "a small fraction of the amino acid chain" can be regarded as "packing or scaffolding and (has) not much more to do with the activity of the protein than had the scaffolding which allowed (the artist) to lie on his back... to paint the ceiling of the Sistine Chapel to do with the resulting artwork." This is probably an over-simplification of protein structure. For example, the enzyme, thermolysin, has a reactive site that is concerned with proteolysis, but the rest of the amino acid chain folds into a three-dimensional structure that permits only hydrophobic substrates to reach the reactive site.

Professor Waddington's discursions range across a wide scope of subjects, among them the evolution of developmental systems, adaptations, altruism, and language; genetic assimilation; canalising selection; the selection of environment *Drosophila* mutants; sexual isolation; archetypes in evolution; a catastrophe theory of evolution; and some interesting musings on "The human animal" and "The human evolutionary system"; and "Does evolution depend on random search?" His analysis of the "midwife toad incident"—a review of Koestler's book—is most entertaining.

The reader will find that Professor Waddington's book contains many fascinating excursions into evolutionary philosophy and into descriptions of the adaptations and special properties that make living organisms so enthralling to all of us. **T. H. Jukes**

MTP announce the publication of

## The Biology of Cancer

A new approach  
by Professor P. R. J. Burch

This book is a learned and closely-argued challenge to many current assumptions about the nature of malignant disease and the mechanisms of carcinogenesis. In particular it questions the widely-held presumption that smoking causes various cancers including lung cancer.

The book expounds and amplifies a theory of cancer and carcinogenesis that was first published in outline in *Nature* in 1970. A wealth of supporting evidence is cited from fields as diverse as molecular biology and clinical medicine.

This theory of cancer is the most detailed and comprehensive yet proposed: it also makes precise and testable predictions as well as suggesting some new approaches to the therapy of cancer.

January 1976 448 pages  
Price: £11.50

MTP Press Ltd.,  
St Leonard's House,  
Lancaster, LA1 1PE

## Evolutionary thoughts

*The Evolution of an Evolutionist.* By C. H. Waddington. Pp. xii + 328. (Edinburgh University Press: Edinburgh, May 1975.) £6.00.

PROFESSOR Waddington has reprinted twelve of his journal articles (five of them from *Nature*), and twelve excerpts by him from textbooks and symposia volumes. To these he has added two exchanges of correspondence (with T. Dobzhansky and P. M. Sheppard) and four book reviews. Two brief essays, not published elsewhere, are also included. The time span of the material reprinted covers from 1941 to 1974. Some of the subject matter is concerned with *Drosophila* genetics, and there is also a great deal of discussion of the differences between various trends of evolutionary thought.

I have a feeling that the question of the inheritance of acquired characters, so much a target of discussion and refutation by evolutionists, was dealt a mortal blow by the discovery of the molecular mechanism of protein synthesis. This point is not mentioned,



UNACUSTOMED as I am to watching television, it was with some diffidence that I agreed to review *The Human Conspiracy*, a programme that is part of a series intended sporadically to bring the latest scientific developments to your fireside. My experience while watching reminded me of those of a wealthy friend who always insisted on travelling first class. He had been brought up to believe that the most terrible goings on occurred in the second class and on the only occasion when he ventured to pass into that part of a ship, his worst expectations were confirmed. He wandered down a gangway and seeing an open door, entered a cabin just in time to cut down a passenger who had attempted to hang himself, doubtless driven to desperation by conditions in steerage. Whether this programme is typical of the BBC or whether it represents an isolated attempt at self destruction, it is not for me to say.

The programme consisted of a series of fragmentary episodes loosely organised around the theme of how men and women get to be the way they are. Viewers were shown psychologists in Belgium posing an impossible problem to a prisoner to assess his reactions, although just how this would help to determine whether he should be released was not made clear. A sequence showing a schizophrenic and her identical twin in Bergen led up to the misleading statement that "for schizophrenia it's two-to-one environment versus genes"—Ladbroke's please note. Malnourished children in Mexico were presented to demonstrate that starvation can lead to irreversible losses in intelligence. Liberian children have difficulty with mathematics, although their mothers are not surprisingly better at judging the amount of rice in a bowl than American college students. X-ray pictures of a foetus in the womb showed that babies are sensitive to their environment long before they are born. This sequence was duly followed by a picture of a baby being born but we were not told what this illustrated. We were then treated to innumerable shots of babies breast feeding, crying, following objects with their eyes, and, of more interest, howling at the sight of three simultaneous images of their mothers.

The second half concentrated on social interactions. Ron Moody donned a long nose to demonstrate that people tend to shun actors wearing false noses. Boys are more aggressive than girls, probably because they have more testosterone: so bring on a male chimpanzee displaying; and to illustrate that self-sacrifice is not merely a human virtue, why not have some soldier ants sacrificing themselves in defence of their queen? Robert Trivers discussed

the concept of reciprocal altruism which purports to explain why it is that men can be kind to people outside their immediate family: "if you scratch my back I'll scratch yours and therefore we shall both live to propagate our genes". Shots of poor whites in Pennsylvania and of Maori children illustrated the problem of being a member of a group whose identity is threatened, and the Trobriand islanders

## Missing connections

*The Human Conspiracy (BBC2, Saturday, December 6) set out to investigate new discoveries and theories about our personal and social behaviour. N. S. Sutherland here reviews the programme with some reservations. The still below shows presenter Caroline Medawar.*



were held up as a society free from class hatred and loneliness, and in which man's status depends on how much he gives not how much he takes, although how anyone gets enough in the first place to go on giving was never explained. Finally, war scenes were used to depict the idea that loyalty to a group may result in savage cruelty to out-groups, and the programme ended with the thought that "More than brotherly love, it's courtesy that makes the world go round".

Miss Caroline Medawar was the comère and very school-marmish she was: she gave the impression of having learned her lines carefully by heart and it is not clear whether her stilted intonation arose through failure to understand them or because she wished to dissociate herself from their silliness: she should perhaps be given the benefit of the doubt. The male commentators were more fortunate since they were spared the embarrassment of actually having to appear. Much of the visual material had little to do with the intellectual content and on the principle of shooting whatever moves, shifts to new locations were frequently accompanied by shots of motor cars.

I appreciate the difficulty of devising

a scientific programme intended for a general audience. The most successful attempts, to my mind, have been programmes introduced and informed by a single scientist with sufficient charisma to project his own personality: one example is the late Professor Korner's excellent series on molecular biology. Even where the contents are rather scrappy, as in Bronowski's *Ascent of Man*, the glimpse of an individual mind at work does something to provide a sustaining connection. I suspect that the producers of *The Human Conspiracy* have underestimated the intelligence and staying power of a general audience: a programme that concentrated on a single theme and made explicit the conclusions reached and the research methods used would surely be more gripping than one that makes a series of disconnected points none of which are developed in depth.

Moreover, the practice of taking all the examples from ongoing research is misleading, and gives the viewer a spurious impression that he is being presented with new conclusions: we were given no hint that research on in-groups and out-groups has been under way for many years and I could not grasp what new findings were expected to emerge from much of the current research shown. By limiting the programme to such research, the producers lose the opportunity to choose the study that best proves a point, where that study, as is usually the case, has already been completed. Finally, there can be no excuse for perpetrating scientific howlers. It was argued, for example, that since homosexuals cannot transmit their own genes, they only survive because they are of use to their immediate kin. There is, however, no evidence whatsoever that homosexuality is an inherited condition, and the argument is therefore vacuous.

I realise that the programme was not intended for readers of *Nature* and the writer, Nigel Calder, once claimed to follow Faraday in thinking that the "generality of mankind cannot accompany us for one short hour unless the path is strewn with flowers". This patronising remark captures the spirit in which the programme was devised, but even if it were true, some effort might have been taken to provide garlands in a healthy state of bloom rather than dead or wilting blossoms. I personally would have preferred a few brambles to relieve the bland and cosy monotony, typified by a sentiment expressed by Professor Jerome Bruner: "Isn't it clever of Nature to arrange that play like most other things in life doesn't work unless there's some fun in it". Ugh. □



# announcements

## Awards

The Award Program in Cancer Immunology of the Cancer Research Institute of New York gave its inaugural prizes in October 1975 to: Dr Hans O. Sjogren, University of Lund, Sweden; Dr Robert J. Huebner, National Cancer Institute, Bethesda, Maryland; Dr Lloyd J. Old and Dr Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York; Mr Edward J. Foley, Schering Corporation, Bloomfield, New Jersey; Peter A. Gorer (posthumous award); Dr Edmund Klein, Roswell Park Memorial Institute, Buffalo, New York; Dr George Klein, Karolinska Institutet, Stockholm, Sweden; Dr Richmond T. Prehn, University of Pennsylvania; Dr Gertrud Henle, Children's Hospital, Philadelphia, Pennsylvania; Dr Robert A. Good, Sloan-Kettering Institute for Cancer Research, New York; Mrs Helen C. Nauts, Cancer Research Institute, Inc., New York; Dr Donald L. Morton, University of California, Los Angeles; Dr Ludwik Gross, Veterans Administration Hospital, Bronx, New York; Dr Werner Henle, Children's Hospital, Philadelphia, Pennsylvania.

The British Pharmacological Society has awarded the Sandoz Prize in pharmacology to Dr J. Hughes of Aberdeen University for his pioneering work on encephalin.

The Dr H. P. Heineken Prize for 1976 has been awarded to Professor L. L. M. Van Deenen of the University of Utrecht for his research on the mechanism of action of phospholipases and on the structure of biomembranes.

## Appointment

Professor Yosef Aloni has been appointed the first Duckwitz Professor at the Weizmann Institute. The chair, for cancer research, has been established by the Federal German government in honour of Georg F. Duckwitz.

## Reports and publications

### Great Britain

Agricultural Research Institute of Northern Ireland. Forty-eighth Annual Report, 1974/1975. Pp. 49. (Hillsborough, Co. Down: The Agricultural Research Institute, 1975.) [411]

Social Consequences of the Energy Situation—Report of a Study Group. (British Association Publication 75/1). Pp. 15. (London: British Association for the Advancement of Science, 1975.) 50p. [611]

An Introduction to Liquid Scintillation Counting; and Solutes and Solvents for Liquid Scintillation Counting. Combined edition. By Dr. J. B. Birks. Pp. 41. (Colnbrook, Bucks: Koch-Light Laboratories, Ltd., 1975.) *Gratis*. [611]

Potato Marketing Board. Annual Report and

Accounts, 1975. Pp. 36. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1975.) [611]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 915:

**The Medical Research Council's Steroid Reference Collection** has recently received a most generous gift from Professor T. Reichstein, Basel, of some four hundred samples from his extensive collection of steroids, which represents the results of forty years' research. Compounds in the androstane and pregnane series will be included in the next list of additions to the Steroid Reference Collection.

A large part of the gift consists of a unique range of derivatives of bile acids, of the types studied intensively for partial synthesis of adrenocortical steroids. These compounds range from the 'natural' bile acids of the C-24 (cholic acid) series, through nor-acids (C-23) and dinoracids (C-22), down to the etianic acids of the C-20 series. Within each series there are representatives of unsubstituted types, 3-, 11-, and 12-monosubstituted types, 3,7-, 3,11-, 3,12- and other disubstituted types, and finally 3,7,12-trisubstituted types.

Lists of these materials are available to workers who are interested and who can show a direct need for specific compounds; applications should be made to Professor W. Klyne or Dr D. N. Kirk at Westfield College, Hampstead, London NW3 7ST.

The amounts of material available in some cases are small, and many samples represent a considerable part of the world's supply of the compound. In these circumstances the Steroid Reference Collection must exercise restraint in the issue of such valuable materials.

**The Foulkes Foundation** invites applications for its 1976 fellowships. They are intended to finance science graduates taking a medical degree before doing medical research, or, similarly, medical graduates needing an additional science degree. Those interested should contact D. W. FitzSimons, Secretary, The Foulkes Foundation Fellowships, The CIBA Foundation, 41 Portland Place, London W1N 4BN.

A Discussion of the Physics and Chemistry of Biological Recognition. Organized by D. C. Phillips, and G. C. Radda. Pp. 1-198 + plates 1-3. UK £8.40; Overseas £8.65. Vol. 272, No. 916: Early Seed Development in the Triticeae. By M. D. Bennett, J. B. Smith and I. Barclay. Pp. 199-227 + plates 1-3. UK £1.60; Overseas £1.65. (London: The Royal Society, 1975.) [611]

A Bibliography of Geodynamics—oriented Publications by Scientists in the United Kingdom, 1970/1975. Pp. 38. (London: The Royal Society, 1975.) [611]

Wildlife Conservation and Lichens. By O. L. Gilbert. Pp. 16. (Exeter: The Devon Trust for Nature Conservation, Ltd., 1975.) [711]

Land Economy: An Education and a Career. By D. R. Denman. (A Lecture delivered at the 137th Annual Meeting of the British Association for the Advancement of Science, 1975.) Pp. 32. (Berkhamsted, Herts: Geographical Publications, Ltd., 1975.) 50p. [711]

Tobacco Consumption in Various Countries. Edited by P. N. Lee. Compiled by M. J. Wilson. Pp. 86. (Research Paper 6.) 4th edition. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1975.) [711]

Professional Engineers, Scientists and Technologists in the Engineering Industry. By Muriel Venning. Pp. 150. (Research Report No. 4.) (Watford, Herts: Engineering Industry Training Board, 1975.) [711]

Research Supported by the Social Science Research Council, 1975. Pp. 433. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1975.) £1.50. [1011]

The Peak District National Park: The Way Ahead. Pp. 12. (Bakewell, Derbyshire: Peak Park Planning Board, 1975.) [1111]

An Introduction to the Open University. Pp. 24. (Milton Keynes: The Open University, 1975.) [1111]

The Western European Energy Economy: Challenges and Opportunities. By Professor Peter R. Odell. (The Stamp Memorial Lecture delivered before the University of London on 13 November 1975.) Pp. 40. (London: University of London, The Athlone Press, 1975.) [1211]

Commonwealth Bureau of Nutrition. Protein-Calorie Malnutrition of Early Childhood: Two Decades of Malnutrition. A Bibliography compiled by E. F. Patrice Jelliffe. Pp. 118. (Farnham Royal, Slough: Commonwealth Agricultural Bureaux, 1975.) [1211]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 917: The Genetics of the Mimetic Butterfly *Hypolimnas bolina* (L.). By Sir Cyril Clarke and P. M. Sheppard. Pp. 229-265 + plates 1 and 2. UK £2.25; Overseas £2.35. Vol. 272, No. 918: A Discussion on the Results of the 1971 Royal Society—Percy Sladen Expedition to the New Hebrides. Organised by E. J. H. Corner and K. E. Lee. Pp. 267-486 + plates 1 and 2. UK £9.10; Overseas £9.35. (London: The Royal Society, 1975.) [1311]

Wildfowl 26. Edited by G. V. T. Matthews and M. A. Ogilvie. Pp. 176. (Slough, Gloucestershire: The Wildfowl Trust, 1975.) £2.75; \$8. [2011]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 272, No. 919: The Intrinsic, Association and Commissural Connections of Area 17 of the Visual Cortex. By R. A. Finken. L. J. Garey and T. P. S. Powell. Pp. 487-536 + plates 1-21. (London: The Royal Society, 1975.) UK £4.75; Overseas £4.90. [2011]

British Antarctic Survey. Scientific Reports. No. 51: The Geology of the Duse Bay—Larsen Inlet Area, North-East Graham Land (With Particular Reference to the Trinity Peninsula Series). By Dr. N. Aitkenhead. Pp. 62 + 12 plates. £6 net. No. 83: A New Assemblage of Plant Fossils from Milorgfjella, Dronning Maud Land. By Dr. Edna P. Plumstead. Pp. 30 + 15 plates. £2.60 net. (London and Cambridge: British Antarctic Survey, Natural Environment Research Council, 1975.) [2111]

Journal of Thermal Biology. Vol. 1, No. 1, October 1975. Edited by K. Bowler and J. E. Heath. Pp. 1-60. Published quarterly. Annual Subscription Rates 1975: \$35; Individuals whose institution takes out a library subscription may purchase a second or additional subscription for personal use at \$25. (Oxford and New York: Pergamon Press, 1975.) [2611]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 280, No. 1294: Metamorphic Processes at High Temperature and Low Pressure—The Petrogenesis of the Metasomatized and Assimilated Rocks of Carneal, Co. Antrim. By P. A. Sabine. With X-ray Studies by B. R. Young. Pp. 225-269. (London: The Royal Society, 1975.) UK £2.15; Overseas £2.25. [2711]

Chemical Industries Association Limited. Activities Report 1973/1975. Pp. 16. (London: Chemical Industries Association Limited, 1975.) [2711]

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Science Research Council. Advanced Ground Transport—a Review of Research Possibilities. Pp. 73. (London: Science Research Council, 1975.) *gratis*. [2811]

**nature***January 15, 1976*

## Time to speak out against cosy bilateralism

A YEAR and a half ago the governments of the United States and the Soviet Union declared that from March 1976 they would refrain from test-firing nuclear devices with yields of more than 150 kilotons. Atmospheric testing of devices of all sizes has been banned since 1963 (except that France and China never acceded to the treaty) but there has been a marked lack of enthusiasm among the nuclear powers for constraints on underground weapons tests. Now it looks as if even this modest bilateral measure may fail to materialise.

Testing of warheads has never been absolutely vital to a nuclear programme but there are obvious benefits in the regular, full scale firing of devices to try out new ideas. Weapons laboratories would find a total ban, if not unbearable, at least an inconvenience, and up to the present this has prevented really serious talk of a comprehensive treaty, in spite of regular pressure from non-nuclear powers who have seen the superpowers busily involved in 'upwards' proliferation while urging stringent controls against 'sideways' proliferation. For many years the vagaries of seismology were used to rule out a treaty—how could one go into an international agreement without adequate monitoring for violations? It will always be possible to deploy such an argument, of course, by insisting on lower and lower detection levels, but in recent years it has become clear that seismic monitoring capabilities are beginning to settle at a level at which a massive (and most unlikely) investment would be needed for further major advance.

When it became clear that the superpowers, under pressure to show at least some concern for the non-nuclear powers at the 1975 Non-Proliferation Treaty conference, were talking about a limited restriction, it was widely assumed that agreement would be reached on a level of about 50 kilotons (with which seismology could easily cope). When the figure of 150 kilotons was announced, there was general astonishment that anything so weak could seriously be proposed as an arms control measure. In hindsight, anyone who had seen the multi-lateral debates of the 1960s about strong arms control treaties give way to the bilateral SALT talks with their conveniently high ceilings should not have been surprised that the treaty had been designed to minimise inconvenience to the contracting parties. And the run-up to the treaty has allowed ample time for the testing of

larger devices—time which has been well used.

Unfortunately, two serious and entirely predictable problems have hampered the bilateral technical discussions thus far. The first is the difficulty in defining yield with any precision on the basis of remote measurements (the Soviet Union has consistently refused the United States access to any instrumentation on Soviet soil). The geological complexity of test-sites and the strange focusing and defocusing of seismic waves makes for uncertainties in yield determination of a factor of at least two, and the extensive East-West interchange of data in recent months does not seem to have brought much clarification.

The second problem is how to treat peaceful nuclear explosions. They have become very popular in the Soviet Union, which is thus unwilling to talk about even a 150-kiloton ceiling for its peaceful operations. If any doubted that warheads could be tested under a peaceful guise, India will have disabused them of that illusion. Ideally the devices would be supplied through an international agency from a stockpile, but if the International Atomic Energy Agency (IAEA) in Vienna has been approached to fulfil such a role it is being kept very quiet.

The omens are not good for the removal of these obstacles by March, but it is an election year in the United States and this tosses in many imponderables. If President Ford has to fight off a serious challenge from Mr Reagan he will probably wish to show he can be tough in foreign affairs—hence, no blind eye in treaties with the Russians, especially since Dr Kissinger has been so criticised for blind eyes to alleged SALT violations. But if President Ford wins the primary, a gesture of statesmanship before the election might well capture the public imagination (as President Kennedy found to his surprise after signing the Partial Test Ban Treaty).

Meanwhile the other nuclear and non-nuclear powers have to decide whether to raise a fuss in their forthcoming talks in Geneva about this cosy bilateralism. Some countries will. But how about Britain? Is it not time to drop the convention of not speaking against American initiatives, and to expose the treaty as a sham? Many people in high places in the United States might even be glad to have an old ally refuse to endorse a footling measure. □



The EEC countries have begun planning a more coherent approach to the exploitation of their joint energy resources. **Ray Dafter**, Energy Correspondent of the *Financial Times*, reports

## Examining Europe's energy equation

THE SO-CALLED energy crisis of 1973 and 1974 may, in retrospect, be viewed as a blessing in disguise for, if nothing else, it applied the minds of nations to something they had chosen to ignore for decades: the sensible planning of energy resources for the future. Cheap and plentiful crude oil, mainly from the Middle East, had followed supplies of cheap coal as the basic energy provider and it seemed that everyone had been lulled into a false sense of security. As Mr Frank Zarb, the US Federal Energy Administrator, pointed out in London recently, the industrialised world had paid dearly for its mistake.

The first world conference on energy and raw materials, held in Paris in December last year; the establishment of the International Energy Agency (IEA) by the OECD; the bid for a common energy policy within the EEC—all these are signs of a change in attitude to energy. The fact that the Paris conference produced very little in the way of solutions to the international problem is hardly surprising; the issues are both complex and fundamental. Similarly, it is understandable that the IEA and the EEC are making heavy weather of finding common energy policies; after all, member states are only just beginning to get to grips with their individual plans.

But progress is being made. The EEC Nine, for instance, have already agreed on restrictions on the use of oil and gas in power stations and on the maintenance of minimum stocks of fuel in case of another energy emergency. They are now firmly committed to an energy conservation policy and to a programme to replace imported oil with alternative fuels. It is anticipated, for instance, that even if the EEC economy grows at 4% a year over the next decade, conservation measures should keep the growth of energy requirements down to no more than 3%: a saving of perhaps 250 million tonnes of oil equivalent in 1985. (Last year energy consumption in western Europe was down some 15% on earlier forecasts although it is difficult to measure just how much of this was due to deliberate conservation rather than the general economic recession.)

New agreements also emerged from the stormy EEC summit meeting in Rome early in December last year and



*Oil: 'Already there is a feeling in the industry that the plums of the North Sea have been picked'*

Photo: J. Allan Cash

these could well prove to be the cornerstones of future European energy policies. For a start there now seems to be widespread acceptance, both in Europe and in the wider IEA, that there should be a minimum safeguard price for oil—in essence a safety net for producers like the UK and Norway should world prices tumble.

Second, Mr Harold Wilson's apparently ignominious stand-down from his demand for a separate seat at the Paris energy talks did persuade France to back an oil-sharing policy in times of crisis: surely a vital principle in any unified energy policy.

Now comes the task of putting shape and meaning to these general policies. The European Community has, as a basic aim, the intention of cutting its dependence on oil imports from the present 60% of energy requirements to around 50% and possibly 40% by 1985. Here the North Sea must make a timely and important contribution (which was why Britain was seeking its separate seat at the energy talks in the first place). By 1980 Britain should be producing between 100 million and 130 millions tonnes of oil a year, making it the only country in the EEC self-

sufficient in energy. By 1985 Britain's North Sea oil could account for around 45% of the Community's total energy output; put another way North Sea oil and gas could, by 1985, be satisfying up to 12 to 15% of the Community's energy needs. In addition, the North Sea could be providing a privileged source of imports from the Norwegian sector worth perhaps a further 5-7% of needs.

This is where the importance of a minimum safeguard price comes in. By 1980 some £5,000 million is likely to have been spent on oil exploration and development in the UK sector of the North Sea. This money (plus a great deal more on ancillary facilities) is being invested on the assumption that oil prices will remain high and provide a reasonable return. If for some political reason Middle East producers decided to slash the price of their oil—and their production costs are a fraction of those in the hostile North Sea—then Britain and the exploration companies will find themselves in deep trouble. As it stands, the 18 members of the IEA have agreed in principle to a floor price of \$7 a barrel, more than \$4 below the present market price but sufficient, in the Agency's eyes, to protect the development of alternative sources of energy.

And this is what it is all about—reducing the industrialised world's dependence on OPEC oil and encouraging the development of other energy providers. Here, the North Sea provides a useful stop-gap, but it would be foolhardy at this stage to over-emphasise its long term importance. Even on British Department of Energy estimates, reserves from proven fields are likely to last for only 10 to 20 years, though, of course, new fields are being discovered at an encouraging rate.

Professor Peter Odell, Director of the Economic Geography Institute at Erasmus University, Rotterdam, and an often-quoted authority on energy matters, feels that present and future trends in the North Sea offer "the possibility of a long term supply of indigenous oil capable of bringing the dependence of western Europe on foreign oil down to almost a derisory level."

Undoubtedly new commercial fields will emerge in the North Sea over the



next decade (a new licensing round in the British sector is planned for later this year). How commercial they prove to be will depend on their size, the ease of recovery and the closeness to existing fields (which should help transporting costs), as well as on the price of oil. Already there is a feeling in the oil industry that the plums of the North Sea have been picked.

In the short to medium term, oil and natural gas must inevitably play a dominant role in the EEC's energy use—around 64% of total use if the Community's plans come to fruition, a large proportion of these hydrocarbons coming from the North Sea. The importance of natural gas should not be underestimated—the EEC hopes to obtain at least 175 million tonnes of oil equivalent from member countries by 1985; if possible some 225 million tonnes. But what happens when these valuable resources start to dry up? It would be fanciful to predict that some of the more colourful ideas will fill the gap. True a lot of research is going into harnessing energy from waves, tides, the wind and the Sun. On paper many of these possibilities look attractive. For instance, the energy theoretically available from waves is immense—the equivalent to about 70 kW per metre on the Atlantic-facing coastline of Britain alone. The sheer cost and technical problems put such schemes well into the future, however.

Even if one adds in geothermal heat, which has received so much publicity recently, it is unlikely that these newer methods of raising energy will provide more than 6–8% of requirements by the end of the century—at least this is the conclusion of a Department of

Energy 'think tank' (Energy Technology Support Unit) set up in June 1974.

This, then, leaves nuclear power and the 'old faithful', coal, to fill any gap left by a fall-off in oil or gas; or at least to take up much of the energy growth over the next 25 years or so. In some ways this is a daunting prospect, for in Britain this implies an expansion by 20 times of the country's present nuclear capacity by the year 2000; daunting because it poses the problems of technology (which type of reactor should be used), lack of indigenous uranium resources, and public attitudes (the row in the UK over nuclear waste disposal cannot be ignored).

Looking further afield, on present projections nuclear power would need to provide the EEC with 16% of its energy requirements if the Community is to reach 60% self-sufficiency by 1985. This is a big leap from the 1.4% share provided in 1973. The more optimistic projections for the use of nuclear fuel put the amount of installed EEC capacity at 200,000 to 225,000 MW (electrical) by 1985 whereas even the ambitious national plans of member countries go no further than 160,000 MW.

All these problems highlight the folly of writing off the coal industry as outdated and unloved. The coal industries in both the UK and Germany are likely to be given a boost over the next few years. Britain, for instance, has just begun to plan for an expansion programme that could take its output back up to 200 million tonnes a year by the end of the century. (In the past decade output has fallen from that



Europe's Energy Commissioner,  
Henri Simonet

level to around 130 million tonnes a year.)

World resources of all types of coal have been estimated to be about 11 million million tonnes of which 700,000 million tonnes are known to be economically recoverable. But, coal or geothermal power, it is too early to assess with accuracy the relative importance of different forms of power even over the next decade. Policies are still emerging. But at least the industrialised world has learned the hard way that it must make the most of all available resources. It must be efficient in its use of energy, but above all it must be flexible. □

## Turkish poppy industry

### Straw cogs

*The results of Turkey lifting its ban on the cultivation of opium poppies may surprise the sceptics, argues Peter Collins*

THE Turkish Government seems to have scored a major success with its programme to control opium poppy cultivation and hence the illicit production of opium for the world's black market in drugs. Reporting on a visit to the poppy-growing areas at the harvest season, a United Nations official responsible for advising the Turkish government said that "no evidence could be found of opium production in Turkey in 1975". This should reassure those who may have had doubts about the Turks' ability to carry out their intentions, and confound certain United States senators and others who in 1974

foresaw America being flooded with heroin (the most dangerous opium derivative) when the Turks lifted their complete ban on opium poppy cultivation—a ban imposed largely because of pressure from the United States.

An important factor has always been that the Turkish farmers in the areas concerned have never grown poppies primarily as a source of opium, but rather for the seed and the oil derived therefrom which has traditionally been used as a normal part of their way of life. The Turkish government has prevented disruption of the local farming system by allowing the farmers to grow the poppies, though only on registered, and limited, areas. At the same time it has forbidden the lancing of the fruiting capsules, which must be done to get the opium. By harvesting the poppy straw (as the rest of the plant is called) as a monopoly, the government has also been able to continue supplying the world's pharmaceutical trade with the

raw material from which codeine, and hence various other materials, are extracted. All this has helped avoid a world shortage of codeine as well as cut off a potential source of illicit heroin.

No one expects a major programme like this to be a total success immediately. At least one of the problems raised could hardly have been foreseen. During an inspection tour of the growing areas, the UN team found that the government measures were if anything being too harshly applied. The areas that farmers had been allowed to sow were laid down in hectares, and a considerable number of farmers were found to have sown more than the area for which their licences allowed. The local farmers, it seems, think in terms of the dunum, an area which is actually slightly larger than the decare (1/10th of a hectare). Although this was a genuine mistake, understandable in a country where almost every region has



a local system of weights and measures, the law was extremely harsh on these men. Nor could any respite be obtained for them when the UN team pointed out that the excess area planted was in fact very small, and that officialdom as much as growers had made a mistake. The official argument has been that the arrest of the offenders will emphasise that the government really does mean business, and that to release them will bring the law into disrepute. Each must wait till the law has run its course; but if he can show that a mistake was genuinely made, he will eventually be released and granted a licence to grow poppies again, which would not otherwise be the case.

Meanwhile, the area any one farmer may cultivate has increased to a maximum of 5 decares from the permitted area in 1975 of 2 decares, which many farmers considered was too small to

be worthwhile. But it looks as though most of the farmers who have suffered arrest will not have had their cases heard in time for this year's sowing.

As far as the mechanics of the control system are concerned, the government programme seems to have been well organised and implemented. A number of 5-man teams were set up to inspect and control cultivation, and they are backed by 80 teams of gendarmes for detailed inspection. Vehicles for these teams have been provided through UN funds, and the same source of finance is being used to set up a telecommunications network between such major centres in the poppy-growing area as Afyon and Konya and the mobile inspection teams.

Other UN assistance is directed towards providing equipment for laboratories in Turkey for morphine

detection, carrying out training in this field at the Narcotics Laboratory in Geneva and training law enforcement personnel. The funds have also been used initially to underwrite a good guaranteed price for farmers. In 1975 they received 17 Turkish lira a kilogram for poppy "straw" and retained the seed which is their main interest. Although research is in hand to evolve more productive varieties of poppy in terms of both seed and morphine content, it is also hoped that eventually more farmers will turn to other crops, in accordance with official plans for the overall development of the areas concerned. Finally, plans are well advanced, again with UN advice and assistance, for the establishment within Turkey of a processing plant to handle the poppy straw which is now the main export product of the former opium-producing region. □

THE energy plan proposed last summer by the Minister for Industry, Sr Donat-Cattin, was approved on December 23 by the CIPE, the inter-ministerial committee for economic programming. The crux of Sr Donat-Cattin's plan is to supplement the three nuclear power stations already in existence with twenty or more by 1985, which will double the country's electricity production capacity.

In the heated debate over the plan this autumn, one of the most frequent criticisms has been that Italy will simply replace its dependence on the oil-producing countries with one on the uranium-producing countries, without gaining anything. It is also felt that the tremendous increase in capacity, which assumes growth of 4-5% a year, is unnecessary. The electricity board (ENEL) has countered this by pointing out that in the sharp recession of 1975 consumption decreased by only 1% of 1974 consumption, and claims that its plans are flexible enough to be adjusted in the future if necessary.

The US companies Westinghouse and General Electric are to build the 1,000 MW water-cooled reactors, and this has not pleased the Italian trade unions. Italian companies would, in fact, have been capable of doing a large part of the construction work, greatly relieving the balance of payments burden and the unemployment problem. And of course there are the worries about the safety of the US reactors.

A miniature and more furious version of the national debate has been going on in Lombardy, where Sr Donat-Cattin wants sites for two of the reactors to be found. The

regional government wants only one reactor. Instructed by the central government to find one site on the Po near Mantua and another near Lake Como, it has missed the deadline for doing either. The central government now has to choose.

## Letter from Italy

from Gillian Boucher



● What elsewhere has been reported as Italy's blocking of the EEC project for nuclear fusion research has been seen in Italy as resistance by the other EEC countries to the Italian site for the project, Ispra. The EEC Commission felt Ispra to be the most suitable site for the experimental thermonuclear plant, the Joint European Torus (JET); led by the Minister for Science, Sr Pedini, to believe that the decision was virtually final, Italians were startled by the inability of the research ministers to agree about it when they met in Brussels in December.

As enumerated by the Commission, the advantages of Ispra over its competitors—the chief of which is

Culham in the UK—include its connections with Euratom, abundant electricity, available skill, experience of safety measures, and a pleasant environment (on the shores of Lake Maggiore) with an international school. Fears that work may be interrupted by labour problems seem exaggerated; Ispra has suffered industrial action in the past, but in 1975 only 34 hours were lost through strikes. The Euratom link, however, is not an entirely positive attribute: Ispra has constantly suffered from a lack of proper organisation and last summer faced a crisis which only a special grant of 2,000 million lire from the EEC averted. If it does not win JET its future looks extremely uncomfortable.

● After centuries of shooting anything that flutters or cheeps, the Italians have suddenly become wildlife-conscious. A bill on hunting has just been approved by the Senate; when the Camera, or House of Deputies, has approved it, it will provide general principles to guide regional governments in drafting their own rules.

According to the bill anyone who wants a gun licence will in future have to pass an examination on the applied biology and zoology of hunting, the law on hunting, firearms and how to use them, and conservation. Italians wishing to shoot will be required to take out third-party insurance, a protection which can hardly make them more carefree about where they point their guns than they are at present. Wildlife reserves will be established, and what may be shot where, when, and in what numbers will be specified in detail.



# correspondence

## The Loch Ness Monster

SIR,—In their recent article entitled "Naming the Loch Ness Monster" (*Nature*, December 11) Scott and Rines propose formal generic and specific names for a rhomboidal object photographed in Loch Ness. They fail, however, to demonstrate with any conviction that the object is animate, that it shares anything with the later photograph showing two very differently shaped images, or that there is any basis whatever for their suggestion that it represents a species of reptile.

One of the great achievements of eighteenth century zoologists was to devise a disciplined system for the description and naming of animals, one result of which was effectively to distinguish between the real and the mythical animals of earlier writings. The code of nomenclature that has been developed over the succeeding years has been very carefully designed to adjudicate only with regard to the choice of names, thereby avoiding any restriction of freedom in the interpretation of zoological evidence. The onus is therefore on authors and editors to maintain standards of description and rational argument to prevent a return to the days of uncritical mythology.

Readers of *Nature* might reasonably expect an article presenting and interpreting original taxonomic data to have been subjected to the normal refereeing process. The evidence presented for the existence of *Nessiteras rhombopteryx* as a new species of animal falls far short of any normal standards expected in taxonomic zoology, even allowing for the preliminary nature of the report. No details are given of the 'optical data' by which the sizes of the objects were determined, nor of the technique by which the first two photographs were determined, nor of the technique by which the first two photographs were 'computer-enhanced'. No mention is made of controls showing how familiar objects appear on film and sonar traces under the same conditions. These will presumably be included when the observations are published in more detail, but meanwhile it is inconceivable that the application of a name in these circumstances can serve the authors' objective of promoting the conservation of any large animal that might subsequently be found in Loch Ness.

Biologists daily encounter pheno-

mena that they cannot identify or explain. It happens every time a field ornithologist fails to identify a distant bird. He will normally prefer to explain his failure in terms of the limitations of his expertise, the poor atmospheric conditions or the extreme distance, rather than jump to the conclusion that it must be an undescribed species of bird. This analogy is very relevant to a great diversity of so-called unexplained phenomena in Loch Ness.

Zoological taxonomy and scientific publication in Britain have both achieved high reputations. It is a pity to jeopardise these reputations for no good cause. It would be an exciting day for all zoologists if convincing proof were to be produced of a new large animal in the zoologically best explored country on Earth. This paper is unlikely to persuade the scientific community that that day has arrived although it may well serve to mislead the layman into believing that it has.

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### Sir Peter Scott replies:

G. B. Corbet, referring to a rhomboidal object shown in two photographs in the article "Naming the Loch Ness Monster", claims that its authors "fail to demonstrate with any conviction that the object is animate" by which no doubt he means that he is not convinced. Others with equal claims to scientific objectivity have expressed an opposite view, being impressed by the slight difference in the configuration and the orientation of the flipper shown by the pictures, taken one minute apart by a fixed camera on the bottom, which clearly shows that the structure cannot be rigid.

Dr Corbet chooses to ignore the simultaneous sonar evidence with its clear indication that moving objects of large size were present at the time the photographs were taken. He says we have failed to demonstrate "that there is any basis whatever for the suggestion that it represents a species of reptile". We explain that we believe the flipper belongs to a vertebrate animal and that no known aquatic mammal has such a limb. It has not been possible to attribute the shape to any known fish or amphibian; on the other hand, the shape conforms rather closely with the limbs of certain fossil reptiles. In spite

of his analogy which refers to "an undescribed species of bird", it seems unlikely that Dr Corbet would prefer to attribute the Loch Ness Monster to that class. The actual quote from the article is "the inclination is to view it as reptilean".

The objective of the paper was quite clearly stated in the preamble: "Better to be safe than sorry; a name for a species whose existence is still a matter of controversy among many scientists is preferable to none if its protection is to be assured". I do not know what particular expertise Dr Corbet may have for asserting that the objective will not be achieved. Meanwhile it seems sad that he is working so hard to prevent it.

\* \* \*

SIR,—The publication of Scott and Rines and the photographs in the national press indicate that there may be a plesiosaur-like reptile inhabiting Loch Ness. It is exceedingly difficult to envisage how a former tropical marine reptile could endure the cold waters and harsh environment provided by a small lake in Scotland. Since Loch Ness did not exist until some 12,000 years ago, one is faced with the problem of the survival of 'Nessiteras' for a period of 64 million years in a world where its former ecological niche had been occupied by modern cetaceans and pinnipeds.

A certain amount of research on the functional anatomy of plesiosaurs has been undertaken and the results widely reported\*. There were two major types: the long-necked which fed on fish in the surface waters, and the streamlined large-headed forms which fed on cephalopods and were capable of diving to depths of 300 m. The postulated shape of 'Nessiteras' is reminiscent of the non-diving surface living variety; the postulated behaviour is of the large-headed short-necked forms. The evolutionary history of both groups of plesiosaurs can be traced for a period of 150 million years with very minor changes being recorded. It is inherently improbable that from such a stock this strange mixture of both groups would suddenly emerge.

The three key pieces of photographic evidence, which purport to show the neck and part of the body, the right hind flipper and the head, deserve to be analysed in the context of the accumulated knowledge of both living

and extinct aquatic reptiles and other water-dwelling air-breathers. The only way that the neck and body picture could be restored to form a plesiosaur would be to cover a skeleton with skin, but with the musculature reconstructed a different shape would be produced. The appearance of a truncated 'limb' and its negative on the opposite side of the 'body', together with other rectangular areas, would seem to exclude any possibility of this structure being reptilian.

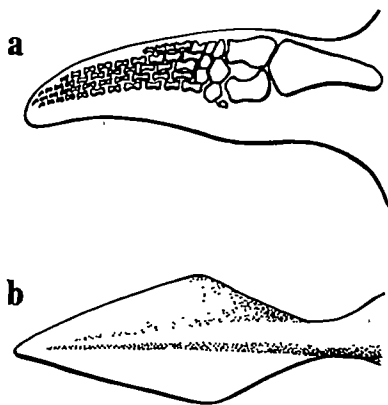


Fig. a Hydrofoil limb of *Plesiosaurus* to show extent of soft tissues (after Robinson 1975).

Fig. b Oar forelimb of '*Nessiteras*' to show axial 'skeleton' (after Scott and Rines 1975).

The correct shape of plesiosaur flippers is rarely illustrated, but it has been known since Dames described skin impressions in 1895. The bones were situated along the leading edge with a tapering fleshy trailing edge; the plesiosaur limb was hydrofoil-shaped (Figs a and b). The limbs of plesiosaurs functioned in the same way as those of marine turtles, penguins and sea lions. The type of fin described by Scott and Rines is not known to exist in any marine vertebrate to our knowledge. It is not a hydrofoil but instead is oar-shaped. There is a central axis and the distal end tapers to a point thus reducing drag. It is inconceivable that an animal with efficient hydrofoil limbs should dispense with them for inefficient oars.

The heads of marine reptiles have their nostrils situated immediately in front of the orbits (the crocodiles are the exception to this but they have achieved the same functional end by evolving a secondary palate). Furthermore, the heads are streamlined. In contrast to this, the photograph of the Loch Ness head has terminal nares with the nasal region being clearly marked off from the orbital by a pronounced ridge or step. There even appear to be horns growing from the frontal region. There is no hint among any group of reptiles of such quasi-mammalian contours.

The evidence claimed to establish

the existence of an aquatic reptile '*Nessiteras rhombopteryx*' allows of an alternative and more logical interpretation.

The 'body-neck' photograph could be of the prow or stern of a Viking ship; the positive and negative projections would be transverse cross-beams of the hull; the longitudinal rectangle would be one of the main planks. It is perhaps worth noting that there are records of Viking raids on ancient settlements in the region of Loch Ness, for example Iona.

Mr Sheridan has already pointed out (reported in *The Times*) the similarity of the 2m long right hand fin to the steering rudder of Viking ships, which is always situated at the starboard stern (posterior right-hand side). Finally the head photograph is exceedingly similar to the dragon heads with which the Vikings embellished the prows of their vessels (and royal furniture). The Loch Ness head would appear to be generically related to the Oseberg head in Oxenstierna's *The Norsemen*.

The features attributed to the new taxon *Nessiteras rhombopteryx* are inconsistent with the anatomy and inferred functioning of any group of extinct reptile. The conclusion seems inescapable. Scott and Rines have discovered the remains of a Viking ship and have mistakenly interpreted them in terms of a living organism.

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\* Watson, D. M. S., *Proc. zool. Soc. Lond.*, 885 (1924); Tarlo, L. B., *Palaeontology*, 1, 193 (1958), *ibid.*, 2, 39 (1959); *New Scientist*, 1414 (1960); Newman, B. H., and Tarlo, L. B., *Animals*, 10 (2), 61 (1967); Halstead, L. B., *The Pattern of Vertebrate Evolution*, 132 (Oliver and Boyd, Edinburgh, 1969); Halstead, L. B., and Middleton, J. A., *Bone bones—an exploration in art and science*, 29 (Oliver and Boyd, Edinburgh, 1972); Robinson, J. A., *N. Jb. Geol. Paläont. Abh.*, 149, 286 (1975); Halstead, L. B., *The evolution and ecology of the dinosaurs*, 68 (Peter Lowe, London, 1975).

Sir Peter Scott replies:

Your correspondents Halstead, Goriup and Middleton argue interestingly that plesiosaurs were either long-necked and lived in shallow water eating fish, or short-necked and dived to 300m to catch squids. They claim that the behaviour of *Nessiteras* (of which we know very little) living in a loch that is 300m deep, but being long-necked, is "a strange mixture of both groups" and therefore "inherently improbable." But in many animal orders whose evolution displays adaptive radiation we find primitive types surviving among the more advanced. To discover features of two known groups combined in one species does not necessarily postulate reticulate evolution.

I agree with your correspondents when they say "it is inconceivable that

an animal with efficient hydrofoil limbs should dispense with them for inefficient oars." But how do we know that the ancestors of *Nessiteras* ever had efficient hydrofoil limbs, and who is to measure the efficiency of their diamond-shaped flippers against the functions they have to perform? Evidently it is adequate for their mode of life.

It may be worth remembering that scores of species of some 53 genera of plesiosaurs are known to science from their fossil bones. In none of them is the skin contour of the head recorded in the stone, and only two examples show the skin contour of the flippers. In many cases the shapes of both birds' wings and fishes' fins vary widely within a single order.

Nor should we forget the processes of convergent evolution. Our paper describing *Nessiteras* said of the flipper "the inclination is to view it as reptilian." Nowhere in the paper was the name plesiosaur used.

The theory that the photographs depict the remains of a Viking ship does not fit the facts, even if the vessel were to be drifting round in midwater like a submerged Flying Dutchman. It is quite impossible, within a number of limiting circumstances, for the head photograph to be a stationary object attached to, or resting on the bottom. These limitations include the geometry of the camera, its strobe-flash equipment and the rope from which it was suspended from the boat, the distance and nature of the bottom below, and the turbidity of the water. On the other hand the Dragon head from Oseberg which they show may, in spite of its mammalian connotations, perhaps have been influenced by monsters well known to the Vikings.

The interpretation of the two pictures of the flipper as a rudder of a Viking ship is perhaps a measure of the inadequacy of modern newsprint reproduction. In the enlargements of the computer-enhanced photographs it is especially interesting that, in the interval of one minute between exposures in a camera standing stationary on the bottom, the flipper has changed shape and orientation. The changes are entirely consistent with the movement of an animal's swimming limb, and could not conceivably have happened if the object had been fixed and solid. These photographs were taken simultaneously with the moving objects shown in the sonar trace published with our article, which seem to have been conveniently ignored by your correspondents. They end with "an inescapable conclusion" from which they might do well to escape after all. If they are interested in Viking ships they will have to go elsewhere to find them.

# news and views

## Terminators and attenuators

from Andrew Travers

POSITIVE control of early gene expression in phage  $\lambda$  requires the function of  $\lambda$  N protein. This regulator permits the transcription of  $\lambda$  early genes yet its sites of action are clearly distal to and distant from the early  $\lambda$  promoters. There is, however, a striking correlation between these sites and transcriptional terminators whose efficient function *in vitro* requires the protein  $\rho$ . From this evidence Roberts (*Nature*, **224**, 1168–1174; 1969) proposed that N antagonised  $\rho$  function and thus allowed these termination signals to be overridden with a consequent extension of the RNA chains initiated at early promoters.

A further insight into the role of  $\rho$  *in vivo* came with the observations that there are  $\rho$ -sensitive terminators within the *gal* operon and that mutant *gal* operons containing polar insertions are also polar *in vitro* in the presence of  $\rho$  (de Crombrughe *et al.*, *Nature new Biol.*, **241**, 260–264; 1973). This suggested that natural polarity of bacterial operons might be a consequence of  $\rho$  function. Another example of transcriptional polarity, that induced by nonsense and frameshift mutations, can be suppressed by second site mutations within the *suA* gene. These mutations, which are recessive to the wild type allele, partially relieve the polar effect of the original mutation without altering the termination of protein synthesis induced by that mutation (Morse and Primakoff, *Nature*, **226**, 28–31; 1970). This parallel between the *in vitro* effect of  $\rho$  in inducing polarity and the *in vivo* effect of *suA* mutants in relieving polarity raised the possibility that  $\rho$  might be the product of the *suA* gene.

Earlier this year this possibility received strong support from observations of Richardson *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1725–1728; 1975). They found that  $\rho$  factor purified from a strain containing the *su78* mutation in the *suA* gene has altered termination function. This  $\rho$  factor fails to terminate transcription at some sites that are efficiently recognised by  $\rho$  factor purified from an isogenic wild type strain. Moreover, a second

*suA* mutation reduces the recovery of  $\rho$  activity from crude extracts by more than 90%. These results do not in themselves prove that  $\rho$  is the product of the *suA* gene, since mutation in that gene could indirectly affect  $\rho$  activity. They do show, however, that termination factor activity is altered in strains containing a polarity suppressor thus again implying that  $\rho$  is involved in transcriptional polarity.

The connection between  $\rho$  function and polarity allowed two predictions to be made. First that polar mutations in bacterial operons might be overcome by active  $\lambda$  N protein. Second that  $\lambda$  N gene mutants which are unable to grow because of their failure to produce sufficient  $\lambda$  early mRNA should themselves be rescued by polarity suppressors. Both predictions have now been verified. N gene function overrides polar mutations in the *gal* and *trp* operons (Adhya *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2534–2538; 1974; Franklin, *J. molec. Biol.*, **89**, 33–48; 1974; Segawa and Imamota, *J. molec. Biol.*, **87**, 741–754; 1974) but only when transcription is initiated at a  $\lambda$  promoter *in cis* to the bacterial genes. This implies that N function requires a phage specific site in order to alter the properties of the transcription complex. The experimental approach to test the second prediction was the direct selection of bacterial mutants which specifically suppress  $\lambda$  N mutants (Brunel and Davidson, *Molec. gen. Genet.*, **136**, 167–180; 1975). These bacterial mutants, designated *sun*, map very close to the known map position of *suA*. Moreover the *sun* mutants are themselves able to suppress polarity in a bacterial operon, albeit only weakly. Conversely one *suA* mutant exhibits a *sun* phenotype. All these results suggest that *sun* and *suA* are allelic. If the only major role of N protein is to antagonise  $\rho$ , *suA* gene function is thus again linked to  $\rho$  activity.

To what extent is the antagonism of transcriptional termination by N protein a specific example of a more general mode of controlling gene expression? Two recent findings suggest that the phenomenon may be wide-

spread. Roberts, together with Sklar *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1817–1821 and 3300–3304; 1975), has provided evidence that the synthesis of  $\lambda$  late mRNA may be controlled in a similar manner to early  $\lambda$  mRNA. They show that a transcription terminator occurs between the promoter for late gene expression and the late genes themselves. This terminator results in the *in vitro* production of a small RNA species about 200 nucleotides long. Late  $\lambda$  mRNA synthesis is under positive control of gene Q and Roberts proposes that the Q gene product acts in a manner analogous to N protein. If this is so it follows that anti-terminator proteins may differ in specificity.

Nor is the positioning of a terminator closely distal to a promoter exclusive to  $\lambda$ . Bertrand *et al.* (*Science*, **189**, 22–26; 1975) have described a strikingly similar structure in the *E. coli trp* operon. The existence of a terminator close to the *trp* promoter was first suggested by the properties of small deletions between the *trp* promoter-operator region and the first structural gene in the *trp* operon. Such deletions produce elevated levels of both *trp* mRNA and polypeptides yet the *trp* repressor-operator interaction is unaffected by the deletion. This effect is *cis*-dominant. Analysis of the RNA produced *in vivo* and *in vitro* from the region immediately distal to the promoter reveals a major RNA species 130 nucleotides long corresponding to the 5'-terminal sequence of the normal *trp* mRNA. *In vivo* the extent of termination resulting in the production of this RNA is regulated. In particular tryptophan deprivation results in relaxation of termination with a corresponding increase in the levels of *trp* mRNA and polypeptides produced. Bertrand *et al.*, propose that the terminator site may function to govern the fraction of initiated polymerase molecules that will transcribe the entire *trp* operon. The control is thus a quantitative modulation rather than a strict on-off switch and accordingly the authors suggest that this DNA signal for termination be termed an 'attenuator'. Other evidence indicates that similar atten-

uators may control expression of the *E. coli ilv* operon and the *S. typhimurium his* operon (Kasai, *Nature*, **249**, 523-527, 1974).

The parallel between the *trp* attenuator and  $\lambda$  terminators overridden by N function is strengthened by the finding that polarity suppressors allelic to *suA* partially relieve attenuation. The original *suA* mutants are, however, relatively inefficient in this respect. Again different alleles of *suA* differ in their effectiveness at suppressing polarity at different terminators. The ubiquitous association of *suA* mutants with a failure to terminate transcription coupled with the selectivity of different *suA* alleles argues that  $\rho$  may indeed be the product of the *suA* gene.

This strong suspicion is confirmed by two observations of Ratner (this issue of *Nature*, page 151). First, affinity chromatography analysis of  $\rho$  protein from different *E. coli* strains reveals that different *suA* mutations affect  $\rho$  in disparate ways: one destabilises  $\rho$ , a second apparently increased the molecular weight of the polypeptide, while others which are presumed to be missense mutations overproduce  $\rho$ . Finally by making use of a molecular weight difference between  $\rho$  isolated from different *E. coli* strains Ratner shows that the  $\rho$  gene is tightly linked to the *suA* locus. Together these experiments make it almost certain that the *suA* locus defines the structural gene for  $\rho$  and not a modifier of  $\rho$  protein.

decreases with increases either in the number of internal modes or in the degree of angular dependence. The computed periods for p modes are in fair agreement with the SCLERA observations, though it is not yet certain how they are excited.

This leaves the problem of explaining the 2 h 40 min oscillation. The observers suggest that it is indeed a fundamental radial pulsation of the Sun. If so, this measurement would upset the established theory of stellar structure and, with it, many astrophysicists. Despite the anomalous neutrino flux, it seems unlikely that the accepted theory could be so far wrong. The theorists prefer to interpret this long period oscillation as a high order gravitational mode, for the g modes have periods that increase with the number of internal modes.

Christensen-Dalsgaard and Gough also review the exciting prospects opened up by these new observations. It should soon be possible to identify the modes directly and to determine their frequencies with great precision. These frequencies can then be used to establish the properties of the solar interior and, in particular, the variation of chemical composition with radius. Such combinations of exact measurements with detailed computations are changing the nature of solar physics. This inversion procedure is similar to that used in deriving the Earth's structure from the frequencies of its normal modes (of which over 1,000 are now known). The structure of the Earth had, however, already been established by seismic observations: in the Sun, though we think we know the relevant equations, the structure has not yet been probed. Solar seismology introduces a new precision into astrophysics. □

## Solar seismology

from Nigel Weiss

THE detection of normal modes of vibration in the Sun provides the first proper check on the theory of stellar structure and evolution. By astrophysical standards, this theory is well established and precise: it predicts the properties of main sequence stars and the qualitative features of their later evolution. These successes are obtained after calibrating the theory to fit the mass, radius, luminosity and age of the Sun, all of which are known. The single other measurement that could be used to check the theory was of the solar neutrino flux. Unfortunately, this is an order of magnitude less than theory had predicted. In the last six months, however, three independent groups have announced the discovery of solar oscillations and the periods of these oscillations offer a means of probing the internal structure of the Sun.

About 10 years ago H. A. Hill set up the Santa Catalina Laboratory for Experimental Relativity by Astrometry, in Arizona, with the aim of measuring precisely the gravitational deflection of light by the Sun. To do so required an accurate technique for locating the edge of the solar disk. The SCLERA group were then able to measure the Sun's oblateness and to show that it was consistent with the observed rotation rate at its surface. In addition they discovered regular oscillatory displacements of the edge of the Sun with a period of about 48 min, together with nine higher frequencies of oscillation.

At the Crimean Astrophysical Observatory, A. B. Severny has for many years studied magnetic fields and velocities in the solar photosphere. He has also compared stellar magnetic fields

with the net field that would be measured if the Sun could not be resolved. In this issue of *Nature* (page 87), Severny, Kotov and Tsap report the result of comparing the line of sight velocity at the solar surface near the equator with that around the poles. This enables them to estimate the radial velocity and they have discovered regular oscillations, persisting for at least 7 months, with a period of  $2\text{ h }40\text{ min} \pm 0.5\text{ min}$ .

This remarkable result has been independently confirmed (page 92) by a group from the University of Birmingham. Brookes, Isaak and van der Raay were attempting to measure the gravitational redshift from the Pic-du-Midi Observatory, using a resonance scattering method. Their results, also reported in this issue, show oscillations with a period of  $2\text{ h }39\text{ min} \pm 2\text{ min}$ . The agreement is far too close to be fortuitous; the methods are so different that systematic errors have to be excluded. Thus the Sun seems also to be pulsating with a period more than three times greater than the longest period detected by the SCLERA group. (Hill's procedure was limited to periods less than about 15 h.)

In a third article in this issue (page 89), Christensen-Dalsgaard and Gough, from the University of Cambridge, attempt to identify the normal modes observed and relate them to those derived from theoretical solar models. Stellar pulsations can be classified as acoustic (p) or gravitational (g) modes. The fundamental mode of radial pulsation for a typical solar model has a period of approximately an hour, while the fundamental quadrupole mode has a period of 46 min. In general, the period of an acoustic mode

## Two more biologically active tetrapeptides

from A. J. S Davies

THE search for chemically defined mediators of interactions between mammalian cells is an arduous process. The lymphocytologists, flushed with success at their identification of T and B cells, have been trying valiantly but, aside from a long list of somewhat risible acronyms (MIF, MAF, SMAF, LIF, LAF and so on) little of repute has yet emerged. The chalone concept has been put forward but is tending to founder on the lack of support consequent upon failure of chemical characterisation of the putative medi-



ators. Thymic hormones and transfer factor are part of this twilight world of 'things' which could be useful, but which would gain more credibility if their identities were convincingly established. As we become more aware of the complexities of cell effluents and become more able to place them in biological perspective then the search and the arguments as to worth and reality will become hotter. How nice, against a background of increasing confusion, to find simple chemically defined mediators of a cell interactional process.

Goetzl and Austen (*Proc. natn. Acad. Sci.*, **72**, 4123-4127; 1975) describe in their paper two sequenced tetrapeptides derived from lung tissue which have the capacity to attract eosinophils. It has been known for some time that eosinophil chemotactic factor of anaphylaxis (ECF-A) could be released from either guinea pig or human lung slices during immediate type hypersensitivity reactions, for example contact with rag weed pollen after passive sensitisation with anti-rag weed serum containing high IgE levels. Further it was found that the material released was preformed in basophils and mast-cell-rich organs. The release was modulated by the levels of intracellular cyclic nucleotides in a similar manner to the regulation of histamine release. ECF-A itself was more effective in attracting eosinophils than neutrophils in the rather artefactual chemotactic chambers used for these quantitations.

Goetzl and Austen caused release of ECF-A from human lung tissue by appropriate means or extracted it from the same kinds of lung. The lungs were surgical specimens and one wonders idly why they were removed. The ECF-A was shown to consist of two tetrapeptides the  $\text{NH}_2$ -terminal amino acid of which was either valine or alanine. These two peptides were then synthesised as was the common tripeptide Gly-Ser-Glu. Both the extracted, released and resynthesised tetrapeptides were shown to have the same high levels of activity and to have a slightly preferential attractive effect on eosinophils compared with their effect on granulocytes. It was further shown that eosinophils which had been exposed to the synthesised peptides became incapable of migrating in response to ECF-A (neutrophils were similarly inactivated).

There are many things of interest in this work. Is the factor really as specific for eosinophils as its name indicates? Do the various lymphocytic regulators of eosinophilia operate by causing release of the factor or do they operate solely by potentiating the production of the appropriate antibody in order that an anaphylactic release

might occur? Are these tetrapeptides mediators *in vivo*? One also wonders what is the functional relationship between ECF-A and tuftsin, the phagocytosis-stimulating tetrapeptide (Nishioka, *et al.*, *Biochem. biophys. Res. Commun.*, **47**, 172-179; 1972). Tuftsin and ECF-A are dissimilar in sequence but the existence of at least three tetrapeptides which have biological activities in relation to granulocytes could indicate that many such molecules exist. □

## The IPCS—a major advance in astronomical spectroscopy

from M. S. Longair

It is now over two years since the first spectra taken with the Image Photon Counting System (IPCS) developed by Alec Boksenberg and his collaborators at University College London caused a major stir in astronomical circles. Advanced electronic and television techniques had been applied to astronomical spectroscopy in a device which counts the numbers of photons in different wavelength ranges and stores this information in a computer. Since then a steady flow of papers containing important results obtained with the system have appeared, in particular two recent papers on the quasar 3C273 and the nucleus of the nearby Seyfert galaxy NGC4151 (Boksenberg *et al.*, *Mon. Not. R. astr. Soc.*, **172**, 289 and **173**, 381 respectively). Both sets of observations were made with the Isaac Newton 98-inch telescope at the Royal Greenwich Observatory, Herstmonceux.

In normal astronomical spectroscopy, the light dispersed by a diffraction grating is focused on to a photographic plate and the information is recovered by developing the plate and measuring features directly from the plate or from some form of isodensitometer tracing. In the IPCS, the dispersed light is focused on to the photocathode of an image intensifier of very high gain. Each photon registered by the image intensifier produces a "spot" of light at the output of the tube which is continuously scanned by a television camera. By electronic processing, this "spot" of light is recognised as a single photon appearing at a single point in the dispersed spectrum. The photons are binned into 1,024 separate wavelength channels spanning the wavelength range of the grating. The television scanning technique enables the infor-

mation on each arriving photon to be transmitted to an on-line computer which stores the information continuously in core. Thus the accuracy with which the spectrum can be observed is limited by counting statistics of the photons detected. In turn this is determined by the cathode efficiency of the image intensifier which in the best modern tubes is better than 20% in the blue.

In practice, the IPCS system is operated in a dual-beam mode, with the object in one aperture and a nearby blank region of sky in the other so that the sky background and the background dark current in the image intensifier can be measured directly. It is normal practice to make two sets of observations with the object and background apertures interchanged to compensate for variations in the sensitivity in different parts of the photocathode. Subtraction of the backgrounds is then trivially performed in the computer. Once the observations are completed, the observer can display the spectrum of the object on a television monitor and plot it on large scale graph paper. Additional facilities include provisions for smoothing the spectrum, removing the sloping continuum to measure precisely the centre wavelength of spectral features and so on. The observer can come away from the telescope with his spectra calibrated and ready for analysis. The spectra shown in the papers on 3C273 and NGC4151 illustrate the effectiveness of the sky subtraction technique, the sky lines having almost completely disappeared from the spectra.

There are many practical advantages in such a system which greatly simplify the process of taking astronomical spectra. The integrated spectrum can be observed "growing" in real time as the observation proceeds since the total counts can be continuously displayed on the television monitor. This is particularly valuable when the objects being observed are too faint to be seen visually through the telescope eye-piece and off-set guiding is necessary. Also as the information acquired is directly stored in the computer, there is no possibility of "forgetting to put the plate in" or "ruining the plates". The system is designed to study faint objects and with the 200-inch telescope, for example, the spectrum of a quasar of 20 mag can be obtained in about half an hour. If the rate of arrival of photons is too great the television scanning system saturates, but since many of the most interesting objects are very faint—quasars, radio galaxies, globular clusters in external galaxies—this is not considered by most astronomers to be a disadvantage.

Since it has come into operation the system has proved very reliable and a

negligible percentage of observing time has been lost through faults in it. A measure of the high regard which astronomers have for this device is the fact that it was used continuously on the 200-inch Hale telescope for a period of over 2 months last year and over 500 spectra obtained. At present it is the most powerful system of its type in the world.

The quality of the spectra displayed in the two papers in *Monthly Notices* speak for themselves. Besides the increased speed with which such spectra can be obtained, the main advantages lie in the determination of the absolute intensities of the lines and in the ease with which broad or weak features in the spectra can be recognised. For example, in the spectrum of NGC4151, over 80 narrow lines can be identified and absolute intensities are quoted for all of them. This much improved data base provides a new challenge to theorists for the construction of models of the line-emitting regions in complete nuclei. Perhaps more striking is the presence of very broad wings on the Balmer lines, the lines of He II and of Fe II. These are all permitted transitions and are much broader than the forbidden lines. The fact that no broad forbidden Fe II emission is observed indicates that the broad spectral features arise in regions where the particle density is greater than  $10^7 \text{ cm}^{-3}$ . This situation is very similar to that observed in 3C273 where the forbidden lines are either very weak or absent. In this respect 3C273 differs from most other quasars.

These papers represent the tip of the iceberg—hundreds more spectra await analysis in many different fields of astrophysics. Systems such as the IPCS will shortly be standard equipment on all the large optical telescopes; for example, Boksenberg and his colleagues are at present building a new version of the IPCS for use on the 4-m Anglo-Australian telescope. Thanks to the development of these devices, many very large scale projects which would have seemed quite impracticable 10 years ago are now within the capabilities of the new generation of telescopes. □

## Madagascar issue settled

from Peter J. Smith

THE geographical position of Madagascar before the breakup of Gondwanaland has been a source of controversy for well over a decade. Before Wegenerian drift began, did this continental fragment lie to the north or

south of its present location? Or has it always been at its present latitude, with only a small eastward drift to take it away from the African mainland and create the Mozambique Channel? The confusion now seems to be over, as palaeomagnetism has provided the definitive answer (Embleton and McElhinny, *Earth planet Sci. Lett.*, **27**, 329, 1975).

The first person to say anything about the ancient position of Madagascar was Wegener himself who placed the island in the large coastal embayment of Mozambique, or about 4° south of its present position. Intuitively (which is simply to say that there is some worth in the Baconian rule of "conformable instances"), this is the obvious place to put it, although there are also some respectable geological arguments for this view as Flores (*Trans. geol. soc. S. Africa*, **73**, 1; 1970) has shown. On the basis of aeromagnetic profiles across the Mozambique Channel, Green (*Nature phys. Sci.*, **236**, 19; 1972) also supported a fit against Mozambique, in which he was followed, on other grounds, by Kent (*Nature*, **238**, 147; 1972).

On the other hand, Darracott (*Earth Planet. Sci. Lett.*, **24**, 282; 1974), taking account of the thin continental-type crust in the Mozambique Channel, concluded that Madagascar has always been at its present latitude but has drifted slightly eastwards. This was a slight variant of an earlier proposal from Flower and Strong (*Earth planet Sci. Lett.*, **7**, 47; 1969) that the island has remained stationary, although Wright and McCurry (*Earth planet. Sci. Lett.*, **8**, 67; 1970) felt that the evidence cited by Flower and Strong could not be taken to exclude eastward drift, arguing instead that Madagascar once lay against Mozambique.

The third and, on the face of it, more radical proposal is that Madagascar has moved about 15° southwards and slightly eastwards, in which case it would have originated against Kenya and Tanzania. This view was first put forward by Du Toit (*Our Wandering Continents*, Oliver and Boyd, Edinburgh, 1937) largely on the basis of matching lithologic units and later supported by Smith (A. G.) and Hallam (*Nature*, **225**, 139; 1970) on the basis of a computer fit of the 500-fathom contour. After carrying out a geophysical survey in the Mozambique Channel, Heirtzler and Burroughs (*Science*, **174**, 488; 1971) also concluded that Madagascar has drifted southwards.

Among the mass of conflicting geological and geophysical evidence, palaeomagnetic data is conspicuous by its absence. It is easy to be wise after the event, but an obvious way of deciding between the three hypotheses

would be to obtain a palaeomagnetic direction for rocks laid down on Madagascar before Gondwanaland split and compare it with African mainland directions for the same epoch. It is true that over a decade ago Nairn (*Overseas Geol. Min. Resour.*, **9**, 302; 1964) obtained a palaeomagnetic direction from the Karroo sediments of Madagascar, but no magnetic cleaning techniques were used and at that time there was no suitable African data for comparison.

Using modern methods, Embleton and McElhinny have now obtained a good palaeomagnetic pole position from Triassic-Jurassic sediments on Madagascar and have compared it, first, with the Triassic-Jurassic African pole and, second, with the African pole for the whole of the Mesozoic (possible because Africa has apparently remained fixed in position during this period). Irrespective of how the comparison is carried out, there is no significant difference between the Madagascan and African poles only if Madagascar was north of its present position during the Triassic-Jurassic. In other words, according to palaeomagnetic evidence there is little doubt that before the onset of Wegenerian drift Madagascar lay off the east coast of Africa adjacent to Kenya and Tanzania.

Having made this central point, Embleton and McElhinny go on to cite the geological and faunal observations in its favour. This evidence is convincing too, although no doubt some puzzles still remain. The fact is, however, that the grounds of argument have now shifted. Any geological 'anomalies' must now be reconciled with Madagascar's northern origin; they can no longer be regarded as convincing evidence against it. □

## Immunoglobulins: constant sequences in the variable region

from Pamela Hamlyn

ANTIBODIES—the vast number of protein molecules which react specifically with foreign substances to trigger the immune response—are complex molecules all very similar in design. Each has two heavy (H) and two light (L) chains. Both H and L chains contain a constant (C) and a variable (V) region with regard to amino acid composition. Analysis of related L chains has shown that different amino terminal regions are associated with the same C region and has led to the suggestion that, unlike all other polypeptides, two genes are needed to code for one polypeptide chain. The number of C region genes

seems to be small but there is controversy over the number of V region genes. These genes code for the enormous number of polypeptide chains which confer specificity on the antibody molecules.

Theories to account for the diversity of the V region genes fall into two main categories. Either they suggest that there is a very large number of genes in the germ line encoding the many V regions, or that the number of genes in the germ line is small but it is amplified by somatic processes such as mutation, recombination or excision and repair with error of the DNA. Despite very many attempts in the years following the publication of these proposals no experimental data has been obtained which clearly establishes one theory over the other. Recent information on the V region of L and H chains indicates that variability is localised in interspersed hypervariable regions. The remainder of the V region (framework segments) show a remarkable preservation of amino acid sequences among related chains.

Capra and Kindt have described a model to account for these observations (*Immunogenetics*, 1, 417; 1975). They propose that the interaction of at least three genes is required to form each immunoglobulin polypeptide chain, the relatively invariant portion of the V region being encoded by a limited number of germ line genes while the hypervariable genes would be coded for by a larger number of genes, perhaps 50–100. By some integration mechanism the hypervariable genes would interact with the basic subgroup genes to form the completed variable region which would then, by a further integration, link to the constant region to form the completed gene for the whole polypeptide chain. This model helps to reconcile some of the conflicting evidence used in support of the alternative theories.

Some recent experiments of Friedenson, Tung and Nisenoff (*Proc. natn. Acad. Sci. U.S.A.*, 72, 3676; 1975) could be used to lend support to Capra and Kindt's model. They isolated antibodies of the same specificity from nine individual mice immunised with the same antigen and used the H chain for amino acid sequence analysis of the first thirty or so N terminal amino acids. They found that the amino acid sequences were all identical. Previous studies of this framework region had investigated amino acid sequences in antibodies pooled from several mice so that occasional amino acid substitutions (arising from somatic mutations) would have been difficult to detect. These results therefore strongly imply that somatic mutations do not occur in the

first framework segment and the authors suggest that this may be extrapolated to apply to framework regions in general. Sequence data on the remainder of the variable region will of course be necessary to see if this extrapolation is justified, and to see how the sequences in the hypervariable regions are related in different individuals. □

## So the coelacanth does bear live young

from a Correspondent

A SHORT paper published in *Science* (190, 1105; 1975) by C. Lavett Smith, Bobb Schaefer, and James W. Atz of the American Museum of Natural History, New York, and Charles S. Rand of the Department of Biology, Long Island University, announces the discovery of five advanced young within the body of a preserved female coelacanth, *Latimeria chalumnae*. Their discovery finally settles a long period of uncertainty as to whether *Latimeria* lays eggs or bears live young.

Since its discovery off South Africa in 1938 the coelacanth has been slow to yield its secrets. It was not until 1952, following wide publicity by the late J. L. B. Smith (who described the first specimen), that a second specimen was captured, this time off the Comoro Islands. This revealed the true geographical range of the species to be this French possession in the western Indian Ocean, and since that date some 80 specimens have been captured—all by native fishermen—despite two expensive multinational expeditions to the Comoros. Most of these specimens have been retained in France, one of the exceptions, however, was the female 1.6 m in length and weighing 65 kg at capture in 1962 that is now in the American Museum of Natural History.



*Coelacanth young with yolk sac*

Despite this apparent wealth of material the key question as to whether these fish lay eggs or give birth to live young remained unanswered. Millot and Anthony (*C. r. Acad. Sci. Paris*, 251, 442; 1960) described a female with eggs in the right oviduct (the left oviduct is non-functional) which appeared to confirm oviparity, as had been suggested by the apparent absence of any copulatory organ in the male, and by their earlier dissections of the urogenital systems and orifices of both sexes. Then a female was found with 19 large eggs in the oviduct, these apparently fully-developed eggs being 8.5–9.0 cm in diameter and weighing around 318 g. This appeared to clinch the matter, and Millot and Anthony's view was held by some as confirmed.

There were heretics, however. R. W. Griffith and K. S. Thomson (*Nature*, 242, 617; 1973) pointed out that osmoregulatory demands would be too great to allow a shell-less egg of this size to develop in seawater. In addition, it seemed hard to believe that a fish of this size, with such a low fecundity could possibly have survived by simply laying tennis-ball sized eggs, even if they were hidden in a rock crevice or a nest. While the adult might have protected them from large predators, they would have been open to attack from the numerous crustacean inhabitants (particularly amphipods) of such a habitat. It would have seemed logical to assume that either *Latimeria* mouth-brooded its developing eggs (as do other marine fish which produce few large eggs), or more likely that the eggs developed internally.

Despite this, so deeply rooted was the notion that *Latimeria* was oviparous, that a recent authority (P. H. Greenwood, *A History of Fishes*, third ed., London, 1975) could write, "It must be assumed therefore that the fertilised eggs are physically unprotected; perhaps there is some form of parental care?"

The recent discovery by C. Lavett Smith and his co-workers thus puts an end to an era of muddled deduction. They report that five advanced young of lengths between 301 and 327 mm, and with moderately large yolk sacs (diameter 80–129 mm) were found in the specimen in New York. The young lie free in the oviduct and *Latimeria* is thus definitely ovoviviparous. Apart from their rather larger eyes and a more rounded head profile they resemble the adult closely. Fins and scales are well developed although they lack the fine teeth on the scales of the adult. Smith *et al.* point out that the female was captured in January (the same month in which females with ovulated eggs were taken) and they suggest from this that gestation may require more than a year.

Interestingly, the recent discovery that coelacanths are live-bearers was anticipated from the fossil record by nearly fifty years. D. M. S. Watson (*Proc. zool. Soc., London*, 453; 1927) reported the discovery of two small specimens of *Holophagus* (as *Undina*) within the body cavity of a larger specimen of this Jurassic fossil. It is pleasant that after this lapse of time Watson's observation on the fossil coelacanth has been confirmed by examination of the only living survivor of the group. □

## The meiotic process

from a Correspondent

A Royal Society meeting for discussion on The Meiotic Process was held in London on December 10 and 11, 1975.

THE meeting explored fully the biological roles of meiosis: the halving of chromosome number in the formation of gametes and the synapsis and subsequent genetic recombination between homologous chromosomes. Opening the meeting, C. D. Darlington (University of Oxford) set the tone by pointing out that the chromosomes do not follow the laws of heredity, they make the laws. Almost every subsequent paper was concerned with one or other aspect of the movement, crossing over or disjunction of chromosomes in the two cell divisions which make up meiosis. The dominant theme was the study by electron microscopy of the synaptonemal complex (SC), an organelle of remarkably uniform dimensions and appearance in a wide range of eukaryotes, which mediates the pairing of homologues at the pachytene stage of meiosis.

Reconstructions of pachytene nuclei from serial sections show that the SCs extend along the whole length of the paired chromosomes (bivalents) and are usually attached at either end to the nuclear membrane. One of the major problems is to explain why bivalents do not become interlocked. S. W. Rasmussen (Carlsberg Laboratory, Copenhagen) showed that at an early stage in pairing in the silk worm, *Bombyx*, interlocking can in fact occur, but is in some way eliminated at a later stage. In this organism there is no crossing over or chiasmata formation in females and a progressive modification of the structure of the SC ensures that the chromosomes are held together until metaphase. D. von Wettstein (Carlsberg Laboratory, Copenhagen) showed that in normal

meiosis, where crossing over occurs, the SC is discarded after pachytene and can accumulate as polycomplex material dissociated from the chromosomes. A small part of the SC remains associated with the homologues at diplotene, probably at the points of crossing over. The importance of the recent discovery of nodules or nodes was emphasised by D. L. Lindsley (University of California, San Diego). These are discrete, densely stained bodies of unknown composition lying within or just outside the SC. Carpenter has obtained very suggestive evidence from *Drosophila* that the nodules in some way mediate or are associated with cross overs. Although a wealth of morphological information is available about the SC, so far almost no chemical work has been done. The major component is protein, probably with associated RNA, but it is completely unknown whether DNA enters the SC very occasionally at sites of crossing over, or at many sites, only a few of which generate cross overs.

Other approaches to the problem of pairing have been made with wheat and other cereals by R. Riley, R. B. Flavell and G. A. Dover (Plant Breeding Institute and Department of Genetics, Cambridge). In a wide range of cytological and physiological studies, the importance of the 5B and 5D chromosomes in controlling homologous and homoeologous pairing has been established. It has been shown by temperature shift experiments and by colchicine treatment that events occur before the premeiotic S phase which are essential for normal chromosome pairing. The favoured hypothesis is that homologous chromosomes are pre-aligned at this stage and that a colchicine-sensitive fibrillar protein is involved. M. P. Maguire (University of Texas, Austin) also presented visible evidence for pre-aligned chromosomes in maize. M. D. Bennett (Plant Breeding Institute, Cambridge) showed that there is a linear relationship between DNA content and the duration of meiosis, which is independent of chromosome number. However, increases in ploidy within a species broke this rule and decreased the time of meiosis.

The importance of mutants in probing meiosis was best shown by the detailed studies of D. L. Lindsley and L. Sandler with *Drosophila*. A very large number of mutants are known which may specifically affect synapsis, crossing over or disjunction at the first or second division. Some are being exploited in further studies of the cross over 'nodule'. Many meiotic mutants also exist in yeast, and P. B. Moens (York University, Ontario) described two which are blocked in premeiotic DNA synthesis, but which nevertheless continue to proceed through meiosis

and form recombinants. One of the highlights of the meeting was the film by B. Nicklas (Duke University, North Carolina). He showed that by physically reorienting bivalents of grasshopper meiotic cells, the direction of movement of the chromosomes of metaphase I was determined by the direction in which the kinetochore (centromere) is pointing. Using the method of Telzer *et al.*, he has demonstrated *in vitro* that this may be related to the spontaneous association of tubulin with the kinetochores.

R. Holliday (National Institute for Medical Research, Mill Hill) discussed the likely mechanism of reciprocal and non-reciprocal recombination at the molecular level in relation to the processes of pairing and the formation of chiasmata. Cross overs between naked DNA molecules must be stabilised to form the chiasmata which hold the bivalents together, whereas non-reciprocal events may be transient. In an attempt to explain the ubiquitous phenomenon of interference between cross overs, he suggested that the SC may contain a limiting amount of a DNA-binding protein which is essential for their stabilisation. A major problem in our understanding of meiosis is the lack of biochemical information. The only systematic study has been made by H. Stern and Y. Hotta (University of California, San Diego) using *Lilium* and *Trillium*. In a review of the advances that have been made, Stern described the properties of the minor fractions of DNA synthesised at zygotene, which may be involved in pairing, and in pachytene, which may be due to repair synthesis during recombination. The latter is absent in the achiasmatic hybrid Black Beauty. Proteins which are synthesised at meiotic prophase and may play an essential role in recombination are a DNA-binding lipoprotein and an endonuclease.

Finally, D. Lewis (University College London) presented in one of the discussion periods an intriguing, although somewhat unrepeatable experiment. He described how in late Summer during the war, a V-1 flying bomb exploded at the John Innes Horticultural Institute, resulting two weeks later in out of season induction of meiosis and blossom in apple and particularly plum trees. It soon became apparent that the denudation of leaves was the main effect of the explosion. The result of this experiment, previously kept secret, indicated that the maturation of meocytes may be under the control of inhibitors, produced in this case by the leaves of the trees. This was certainly one of the most exciting experiments presented, and if published, the Materials and Methods should provide particularly interesting reading. □



# review article

## Scientific plans for deep sea drilling

C. A. Williams\*

*The Deep Sea Drilling Project is about to launch phase IV of its drilling operations, known as the International Phase of Ocean Drilling (IPOD). Plans for the first year of IPOD are well under way and a preliminary drilling schedule for the first four years has been drawn up. At this stage it is appropriate to inform the scientific community in general of these plans and to invite comment and suggestions. As in the past the scientific planning is carried out by the Joint Oceanographic Institutions for Deep Earth Sampling (JOIDES). This organisation, international from the outset, will through its various panels continue to provide scientific advice to DSDP for the IPOD programme as it has to the previous phases of drilling.*

THE drilling project dates from the early 1960s when four major oceanographic institutions in the USA, Lamont-Doherty Geological Observatory of Columbia University, the Rosenstiel School of Marine and Atmospheric Science of the University of Miami, Scripps Institution of Oceanography of the University of California in San Diego, and Woods Hole Oceanographic Institution, formed JOIDES. An initial and successful drilling programme was carried out, under contract from the NSF to Lamont-Doherty, during the summer of 1965 on the Blake Plateau off Florida using the vessel Cardrill I. NSF next awarded a contract to the University of California for an 18-month programme, the Deep Sea Drilling Project (DSDP), in the Atlantic and Pacific Oceans. Phase I of this project, using the drilling vessel Glomar Challenger, began in August 1968. Since then the project has successfully continued through phases I-III, having completed phase III at the end of leg 44 in summer 1975. During this period approximately 400 holes have been drilled and ocean drilling pioneered in all major oceans.

The success of this project springs largely from the parallel programme of research and development into the technical aspects of drilling that has coexisted with the drilling operations, and which has provided both increased sophistication of methods and production in terms of material retrieved. Deeper holes can now be drilled with new improved drill bits, though perhaps the greatest advance is due to the development of the re-entry system, enabling a worn drill bit to be replaced and the hole re-entered for drilling to be continued. The next technical milestone will be the construction of a blow-out prevention system, which will open the way to a range of ocean margin sites presently inaccessible due to the possible encounter of high pressure fluids and gases within the sediments.

### New emphasis during IPOD

IPOD will concentrate on drilling below the sedimentary cover of the ocean floors and intends to penetrate deeper into the ocean floor than ever before. There will also be

a greater concentration on fewer but deeper holes and an entire 56-d leg, or possibly longer, of Glomar Challenger's time will be spent on a single hole, penetrating to a depth of up to 2 km into the sea floor, IPOD I, lasting 4 yr, will continue to use the Glomar Challenger, but during IPOD II, when deep holes into marginal basins and deep-sea trenches are planned, it is anticipated that another drilling vessel, or platform, will be used.

Coupled with this re-emphasis, are changes in the organisation of JOIDES. After the initiation of JOIDES by the four US member institutions, the University of Washington became associated with the drilling project in 1968. During 1974-75 not only did the US membership increase to nine institutions with the addition of the Hawaii Institute of Geophysics, University of Rhode Island, Oregon State University and Texas A & M University, but it is now a formally recognised international organisation with member scientific institutions in the USSR, West Germany, France, Japan and the UK. Financial support for the programme continues to be mainly from the NSF, but with additional contributions from the member nations of IPOD/JOIDES.

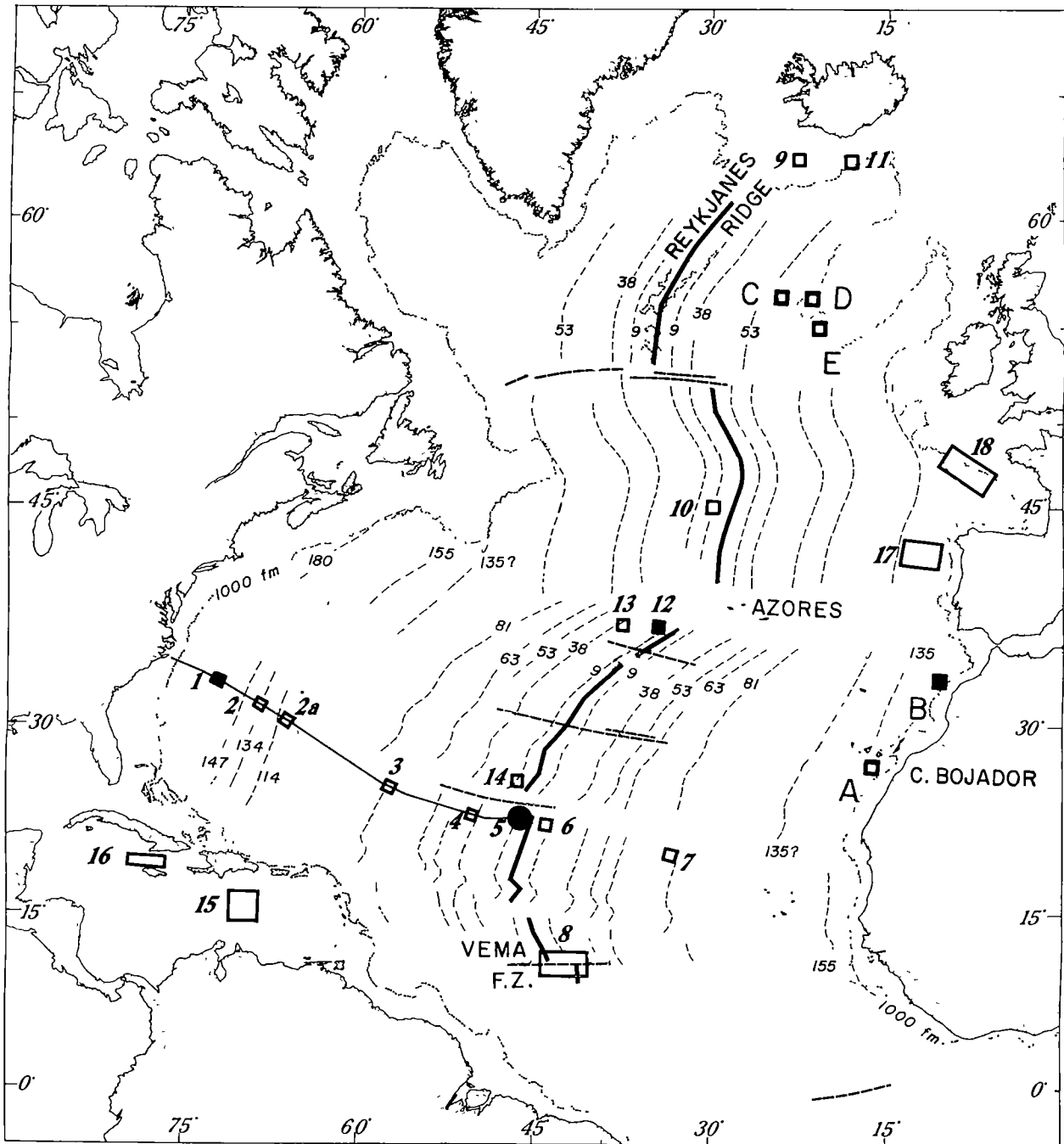
The scientific approach of IPOD differs fundamentally from the previous drilling philosophies in that it is problem, rather than geographically, oriented. JOIDES advisory panels now include four problem panels concerned with the ocean crust, ocean margins (active and passive) and ocean palaeoenvironment.

Since effort will be concentrated on fewer sites, it is essential that the optimum locations be chosen; thus site surveying will also have a considerably more important role than it has during phases I-III. An IPOD Site Survey Management group, situated at Lamont-Doherty Geological Observatory, is designated to coordinate the survey programme and handle subcontracts for IPOD site surveys carried out by US institutions. The input of non-US data into this group remains on a voluntary basis and scientists who have relevant data are encouraged to submit this information for inclusion into the site data package for the chief scientists of that leg.

### Atlantic drilling plans

Five of the first seven drilling legs to be spent in the North Atlantic will be devoted to the study of the oceanic crust. One of the main objectives is to establish a model for the

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**Fig. 1** Proposed IPOD Atlantic drilling sites. Open squares denote single bit sites, filled squares deep sites and filled circles very deep sites. The ages of the sea floor (Myr) are shown along magnetic lineations. The larger boxes indicate the region of several sites not yet precisely determined.

physical, geochemical, petrological, magnetic, thermal and other properties of the oceanic basement and to determine their range of variations, both with time (distance from the mid-ocean ridge crest) and location (along the length of the ridge crest).

The spatial and temporal problem will be tackled by way of two transects. An east-west transect along a 'flow' line (sites 1-7 in Fig. 1) will sample crust that has originated from the same spot on the ridge crest as determined from magnetic anomalies; in this way the variation with age away from the ridge crest will be investigated. The second transect, parallel to the ridge crest, will mainly be on magnetic anomaly 5 West (9-Myr-old sea floor) between latitudes 63°N on the Reykjanes Ridge and 12°N on the Vema Fracture Zone (sites 8-14). Local crustal variability will be investigated by means of shallow trial holes close to each site.

A test for the symmetry in crustal properties on either side of the Mid-Atlantic Ridge will be made by a comparison of samples at sites 3 and 5 with those from sites 7 and 6.

Sites are classified as being either single bit, that is, drilled to the limit of wear of one drill bit, which, depending on the hardness of the rocks encountered, could give a penetration of up to 500 m. Single re-entry sites which will penetrate deeper than 500 m and involve 6-10 d of drilling, and very deep sites involving multiple re-entry, penetration down to 2 km, and take 1-1½ legs of drilling. Atlantic site 5 (Fig. 1) is regarded as a prime site for a very deep hole and will be drilled early in the programme.

The sediments at all holes will be continuously cored and it is hoped that a logging programme can be implemented for some of the holes. Site 10 will be near 45°N. Site 14 is in the trans-Atlantic geotraverse corridor near 26°N; both

are areas well investigated by previous work and requiring little or no additional site surveying. Five holes are planned at Site 8 in the Vema Fracture Zone area. These will be to investigate the nature of the fracture zone and will also extend the longitudinal transect southwards to 12°N.

The original intent of the longitudinal transect was to sample the crust at shallow sites, more or less evenly spaced along the length of the Mid-Atlantic Ridge on magnetic anomaly lineation 5 west. Site 9 will be on magnetic anomaly 5 east and site 11 on the same 'flow' line but on older oceanic crust at anomaly 13 (38 Myr), to investigate the Iceland geochemical anomaly. This represents a trend away from the initial purist approach of avoiding as far as possible any known geochemical anomaly. The change in policy represents both the scientist's inherent propensity to investigate anomalies, or recognition that simple situations only exist where investigation has not yet proved otherwise. As our knowledge of the ocean floor grows, it may become apparent that it is impossible to avoid anomalies. Site 13 will be on anomaly 13 west, along the corresponding flow line west of the FAMOUS area, where, as a result of the very considerable Franco-American effort to study a small area of the Mid-Atlantic ridge crest in great detail, perhaps more is known of the ocean floor than in any other area. Site 12, which will be a deep site near DSDP site 334, and Site 13 will thus form a separate east-west mini-transect in the same way as sites 9 and 11.

Of the first few legs to be spent in the North Atlantic, two of these will concern themselves with the problems of the continental margins. At this stage, four specific areas have been designated: Off north-west Africa, sites A and B, Galicia Bank (area 17), the northern margin of the Bay of Biscay (area 18) and Rockall Bank sites C, D and E (Fig. 1). The first of these sites to be drilled will be site A off Cape Bojador, and a re-entry site in the Morocco Basin. Here it is hoped to sample pre-Oxfordian sediments in the North Atlantic for the first time. Both these holes will also investigate the nature of the sediments relating to the history of this margin during the opening of the Atlantic Ocean approximately 180 Myr ago.

Three sites will be located on Galicia Bank, the submerged continental block which enigmatically does not fit into the reconstruction of the Atlantic continents. The age of the oldest sediments overlying the oceanic crust will be sampled to the west of the bank, and then at a site on one of the faulted blocks on the south or western slope of the Bank, information will be sought on the age and processes involved during the initial rifting apart of the continents. A site on the top of the carbonate platform will investigate the stratigraphy and subsidence history of the platform itself.

Another designated area of interest is the northern continental margin of Biscay, where at three single-bit sites the history of subsidence and sedimentation of this region will be investigated in the light of the history of opening of the Atlantic and Bay of Biscay. Three sites (C, D and E in Fig. 1) are also proposed near the south-west end of Rockall Plateau where the objective is to investigate the history of subsidence of the margin relative to the three different phases of continental separation which have occurred peripherally to Rockall Bank, during the Lower Cretaceous(?), at 80 and 60 Myr.

Additionally, a leg may be devoted to the Norwegian Sea where both crustal and margin problems will be investigated. This programme is still in its initial planning stages, and the proposals for this area will shortly be reviewed by the JOIDES ocean crust and safety panels.

## Palaeoenvironmental aims

The Ocean Palaeoenvironment Panel is interested, through the study of faunas and sediments, in developing a model

for the opening and subsequent evolutionary history of the ocean basin from the point of view of their palaeoclimate, palaeocurrents and so on. The panel also has more specific aims, including a study of the early history specifically of the Atlantic, and to investigate the Cretaceous-Palaeogene boundary as an example of a palaeoecological crisis. Many of their other interests in global ocean circulation, the evolution of plankton communities through time, and so on, require a more extensive global coverage of data than do those of the other panels, and the Palaeoenvironment Panel would like to drill in the Indian Ocean, Antarctic and the South Atlantic as well as in the North Atlantic and North Pacific. There are, however, already more proposals than can be fitted into the programme and even with the recent extension of IPOD I from a 3-yr into a 4-yr programme, it will not be possible to drill in the Indian Ocean or the Antarctic during IPOD I. Two palaeoenvironment legs are planned, however, in the South Atlantic where sections across the Angola and Cape Basins will further investigate the effect of the Walvis Ridge on the early history of sedimentation in the Atlantic.

The development of early plankton communities can be investigated in the northern Pacific, where a hemispherical ocean with an uncomplicated gyral current system is required. Eighteen holes are proposed across the northern Pacific for this programme. The Cretaceous-Palaeogene boundary can be investigated near the Sierra Leone Rise in the Atlantic and also at sites near Rockall Bank, in the Caribbean and in the NW and equatorial Pacific, which will tie in with the other parts of the programme. Volcanic episodicity can also be studied from ash layers in the sediments at sites in the Sea of Okhotsk, the Japan Sea, off Central Chile and also at ocean crust sites near Hawaii and Iceland.

## Pacific drilling plans

Drilling plans for the Pacific are still in outline (see Fig. 2), but the programme will be planned in many respects to parallel the Atlantic work, to compare the slow spreading (half rate of 1–2 cm yr<sup>-1</sup>) Atlantic with the faster spreading (6–10 cm yr<sup>-1</sup>) Pacific. It is not feasible to follow a single flow line across the Pacific as in the Atlantic, and hence the major traverses are split between several flow lines. Two, possibly alternative sites (1 and 3 in Fig. 2) have been chosen to sample very young ocean floor. These candidate sites in the Gulf of California and on the Juan de Fuca Ridge, are both where sufficient sediments to support, or 'spud in', the drill bit occur at the ridge crest. Such sites should provide information on hydrothermal circulation within the crest which may only occur near the ridge crest, on thermal gradients and should also provide unweathered samples for geochemical analysis. The presence of sediments at the ridge crest probably causes an intermixing of lava and sediment and implies that the basement here could be anomalous; however, the value of obtaining very young samples is considered to outweigh this factor.

The fastest Pacific seafloor spreading area, at 20°S, is remote, has a confused magnetic pattern, a thin sediment cover and was rejected in favour of the Siqueiros Fracture Zone at approximately 8°N on the eastern north Pacific (site 4). This relatively fast-spreading transect is planned with sites on 2- and 5-Myr-old crust in sediment ponds existing within 10–20 km of the ridge crest and where seismic refraction data indicate a low velocity zone (possibly the magma chamber?) centred beneath the ridge crest. The spreading rate here was approximately 5 cm yr<sup>-1</sup> over the past 10 Myr, three times that of the Atlantic and adequate to compare the evolution of fast and slow spreading ridges.

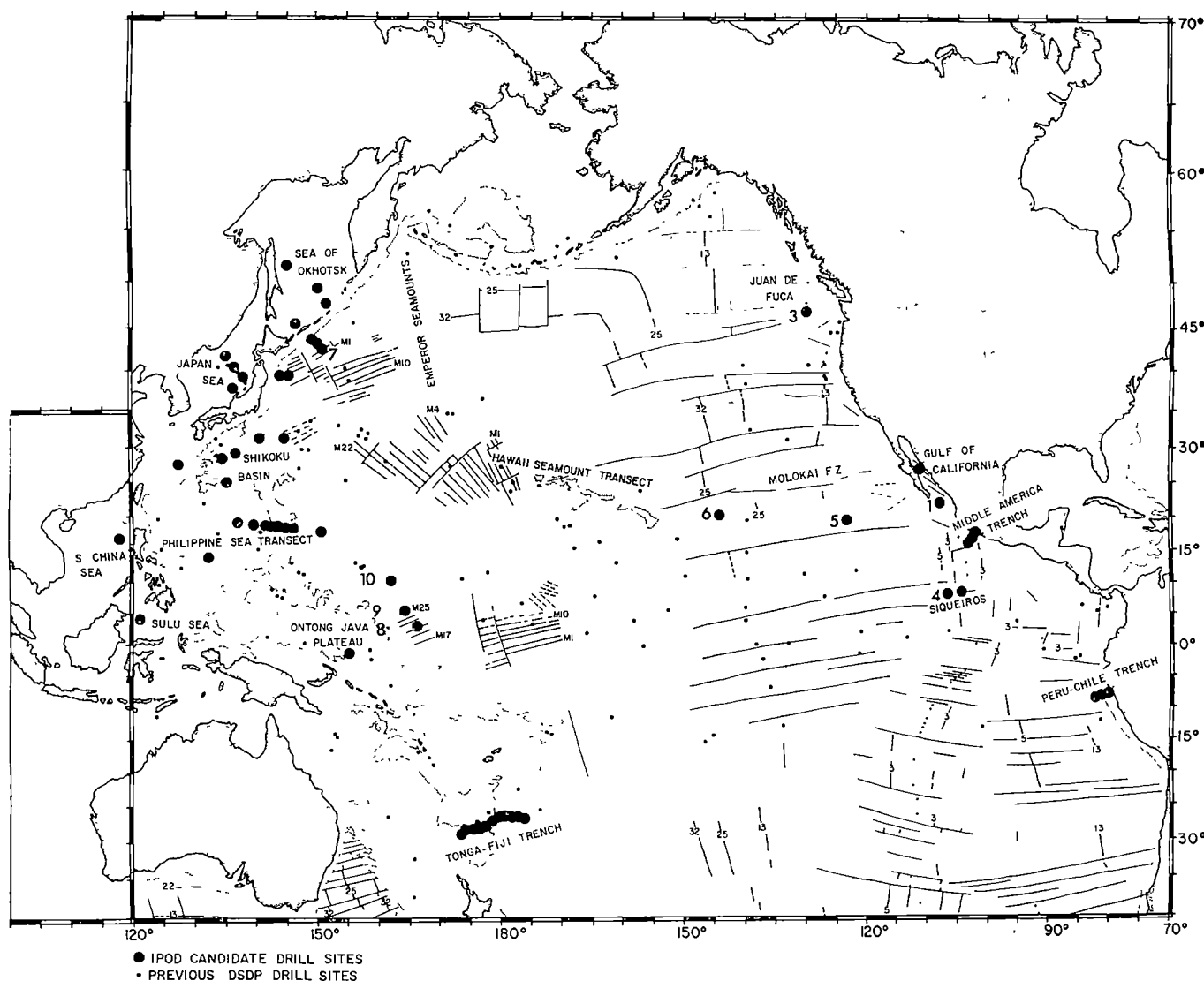


Fig. 2 Proposed IPOD Pacific drill sites. The larger circles denote the proposed IPOD sites and the smaller circles show previously drilled DSDP sites. The multi-site traverses are indicated diagrammatically since the scale of the map does not allow otherwise.

This will be one of the high priority areas for very deep penetration.

Pacific sites 5 and 6 will be shallow holes on anomalies 10 (33 Myr) and 32 (76 Myr) respectively and lie on a flow line south of the Molokai fracture zone which will tie in with the Gulf of California sites.

The oldest known Pacific sea floor and the Jurassic quiet magnetic zone will be sampled at sites 7–10 in the western Pacific. Site 7 will lie on magnetic anomaly M1 (116-Myr-old sea floor) south of the Kurile trench and will date this important magnetic anomaly and also tie in with a traverse from the Sea of Okhotsk across the Kurile trench chosen by the Active Margin Panel. Traverses across the Sea of Okhotsk, the Shikoku Basin and south Philippine Sea are designed to investigate the formation of back-arc and marginal basins. The Philippine traverse comprises thirteen proposed sites with the intention of sampling the floor of the South China Sea, the W. Philippine Sea, the Palau-Kyushu Ridge, the east and west Parece-Vela Basins, the Mariana Islands, trench gap and trench. The results from these sites may allow for clarification between the two current conflicting hypotheses for the origin of the floors of marginal basin areas; that they are areas of entrapped old Pacific sea floor, or alternatively crust formed by sea-floor spreading within the marginal basins themselves.

A transect across the Japan Sea is planned with a series of up to 8 holes to investigate the nature of the crust in that area. All but two of these, and two of the South Philippine transect sites, demand penetration beyond the present capabilities of Glomar Challenger with a 6.7-km long drill string and will be candidate sites for the second phase of IPOD drilling when an 11.0-km drill string is planned. Other active margin traverses are also planned across the Tonga-Fiji trench, the Middle America Trench and the Peru-Chile trench. In due course these will be categorised into priority and contingency sites as the Pacific drilling plan evolves.

The IPOD I programme is scheduled to spend the first eight legs in the Atlantic and Caribbean, the following 12 legs in the Pacific and will return at the end of IPOD I for five final legs in the Atlantic. Clearly the candidate drill sites are already too numerous for all of them to be drilled and the task of the JOIDES advisory panels will be to pare down this number into a workable drilling plan with both established priority and contingency sites.

The JOIDES organisation is keen to hear from the scientific community at large and suggestions are welcome as to precise drilling locations, scientific staffing of the Glomar Challenger for specific legs, and the organisation and allocation of effort with respect to shore-based study of core material, together with relevant existing site survey data.



# articles

## Observations of solar pulsations

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*We have modified our solar magnetograph to measure velocities at the solar surface, rather than magnetic fields. Using this apparatus, we have observed fluctuations of period 2 h 40 min, which are remarkably stable. The interpretation of this phenomenon seems to cause much theoretical difficulty.*

We have reported previously<sup>1,2</sup> on the successful measurement of the Sun's magnetic field using a solar magnetograph and the Crimean Solar Tower Telescope. The magnetograph consists of an electro-optical modulator to pick out the circularly polarised Zeeman components of magnetically sensitive lines, a grating and photomultipliers set on the wings of the lines. We thus achieve sensitivity, but, with the aid of an automatic Doppler compensator, avoid the perturbations of varying relative velocities between the Earth and Sun, turbulence in the atmosphere and apparatus noise. By the use of some additional apparatus we have now investigated periodic fluctuations in the Sun's surface.

### Method and apparatus

To avoid unnecessary complications, we work with a magnetically insensitive line ( $\lambda = 5,123.7 \text{ \AA}$ ). A circular polariser was placed between the telescope and the entrance slit of the spectrograph, of such a diameter to polarise light from the central portion of the Sun only. Light from the solar rim will always reach the photomultipliers (it is initially unpolarised), but light from the central disk will get through only when the magnetograph polariser (modulated at 760 Hz) is set for it. The difference between the outputs of the two photomultipliers provides the magnetograph signal, and the different signals for the two polarisations indicates whether or not light from the centre is shifted relative to that from the rim. The Doppler shift can be converted into a line-of-sight velocity.

To avoid effects arising from the great rotational velocity of the equatorial parts of the Sun, we looked at light from a pole-pole strip across the Sun only. We calibrated the instrument with a line of known intensity and wavelength to relate a known wavelength shift to the measured signal. The interpretation of a positive experimental signal depends on the geometry of the apparatus (the ratio of the area of the polar image to that of the central image) and the mean relative emissivities of the polar regions and central ones.

With our particular geometry, we have derived the following formula for the experimental signal  $\delta_{||}$  in terms of the wavelength shift  $\Delta\lambda_v$ , assuming a triangular line profile

$$\frac{\delta_{||}}{\delta_{||}(O)} = \frac{1}{2+\beta} \frac{\Delta\lambda_v}{\Delta\lambda_c} \quad (1)$$

Here  $\Delta\lambda_c$  is the wavelength shift we produced artificially for the

calibration signal  $\delta_{||}(O)$ , with the aid of the plane-parallel glass plate of the velocity compensator (which is 'off' for calibrations, and 'on' for recording  $\Delta\lambda_v$ ), and  $\beta$  is the ratio of the intensity at the central part of the solar image to that at the rim,  $\beta = I_c/I_r \approx \frac{1}{2}$  in our case (intensities measured by photomultipliers). The calibration is made for a standard shift  $\Delta\lambda_c = \pm 0.031 \text{ \AA}$  of the  $5,123.7 \text{ \AA}$  line, which corresponds to velocity of  $\pm 1,815 \text{ m s}^{-1}$ , so that  $\delta_{||}$  equals  $\delta_{||}(O)$  when  $\Delta\lambda_v = 2.34 \times \Delta\lambda_c$ —for a speed of  $4,238 \text{ m s}^{-1}$ .

### Accuracy of experiment

The accuracy of the method is limited by turbulence in the spectrograph and electronic noise, and can be determined from the accuracy of magnetic field measurements<sup>2</sup>: where mean field strengths of 1 gauss were measured, corresponding to  $\approx 2.2 \text{ m s}^{-1}$ . By increasing the data accumulation time to 15 min, we can achieve  $\approx 1.0 \text{ m s}^{-1}$ , corresponding to a Doppler shift  $\Delta\lambda_v \approx 10^{-5} \text{ \AA}$ .

A special auxiliary guiding system ensured angular accuracy of  $\pm 0.1'' \times 0.2''$  in positioning the beams. There were also effects varying regularly during the whole day connected with slowly changing imbalances in both the electronic channels of the magnetograph, or of photoguiding system, but this slow run of the signal was easily eliminated (especially when we compared the records of two independent magnetographs in our double-channel magnetographic system<sup>3</sup>). In all, 122 h of data (from 21 d) were obtained (August–October 1974 and March 1975).

### Analysis

Analysis showed at once the existence of small fluctuations of signal  $\delta_{||}$  with mean characteristic times grouping around 2 h 40 min. The extrema of  $\delta_{||}$  occurred independently of the local time, and did not correlate with the start of observations (which lasted from 3 to 10 h continuously, depending on weather). This shows definitely that the effect is connected neither with periodic error in the drive of the coelostat nor with the possible heating of optics and misalignment between the main and the guiding system. Moreover, at the beginning of the work we made records of the signal  $\delta_{||}$  in two lines, the solar line ( $\lambda 5,123.7 \text{ \AA}$ ) and the telluric line ( $\lambda 5,901.5 \text{ \AA}$ ), simultaneously using both channels of the magnetograph to check if the effect was real or instrumental. One of these records, presented in Fig. 1, shows clearly that fluctuations arose from the Sun itself. We afterwards used only the solar line  $\lambda 5,123.7 \text{ \AA}$ .

To find the approximate periods of the fluctuation, we overlapped all fluctuations for the first nine days in August 1974 on the same graph to get the best fit (coincidence of maxima and minima) between them (see Fig. 2). This shows an almost sinusoidal wave of period  $\sim 2 \text{ h } 40 \pm 5 \text{ min}$ . We then applied superposed epoch analysis, and folded all data from

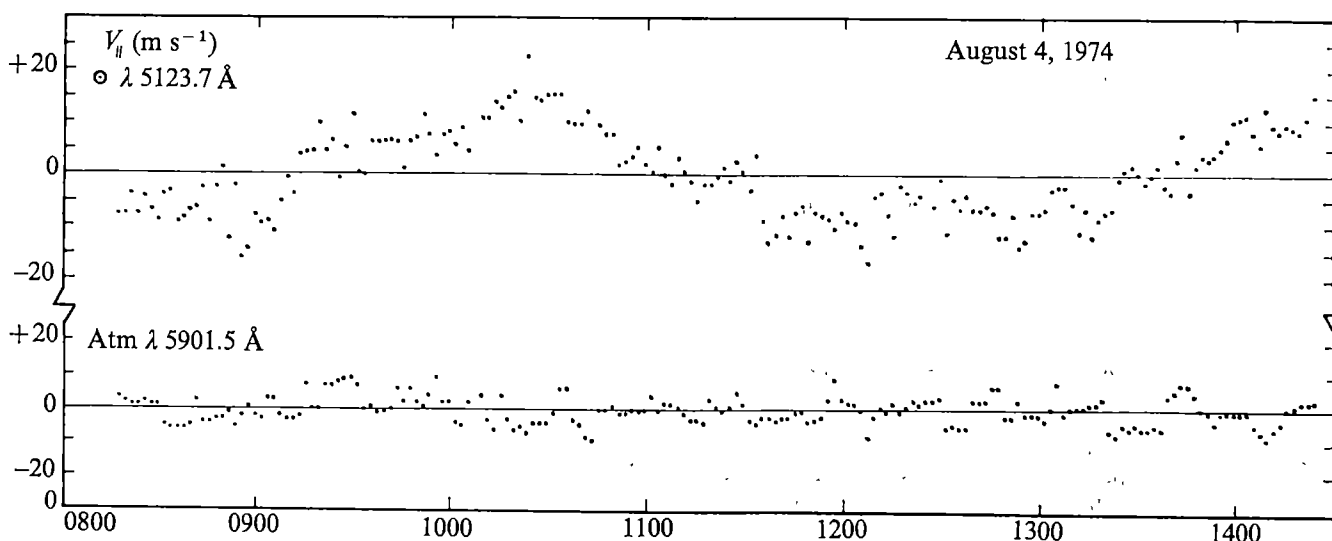


Fig. 1 Measurements of Doppler shift  $\Delta\lambda_v$  made on August 4, 1974, simultaneously on the solar line ( $\lambda = 5123.7$  Å, top) and telluric line ( $\lambda = 5901.5$  Å, bottom). The horizontal axis represents the local time.

August–September, with this approximate period 2 h 30 min–2 h 50 min (with the steps of 0.5 and 1 min) looking for the wave with minimum  $\sigma^2$  deviations. The best fit was for 2 h 40  $\pm$  0.5 min (Fig. 3). The mean amplitude of radial velocity  $v_{||}$  is  $\approx \pm 2$  m s $^{-1}$  so that the amplitude of pulsations is  $\approx 10$  km.

The observations made in October 1974 and March 1975 also showed the same periodicity occurred almost in phase with the data from August–September 1974 (stability over  $\sim 1,900$  periods). Therefore the period can be determined in fact with much higher accuracy than quoted above ( $\pm 0.5$  min).

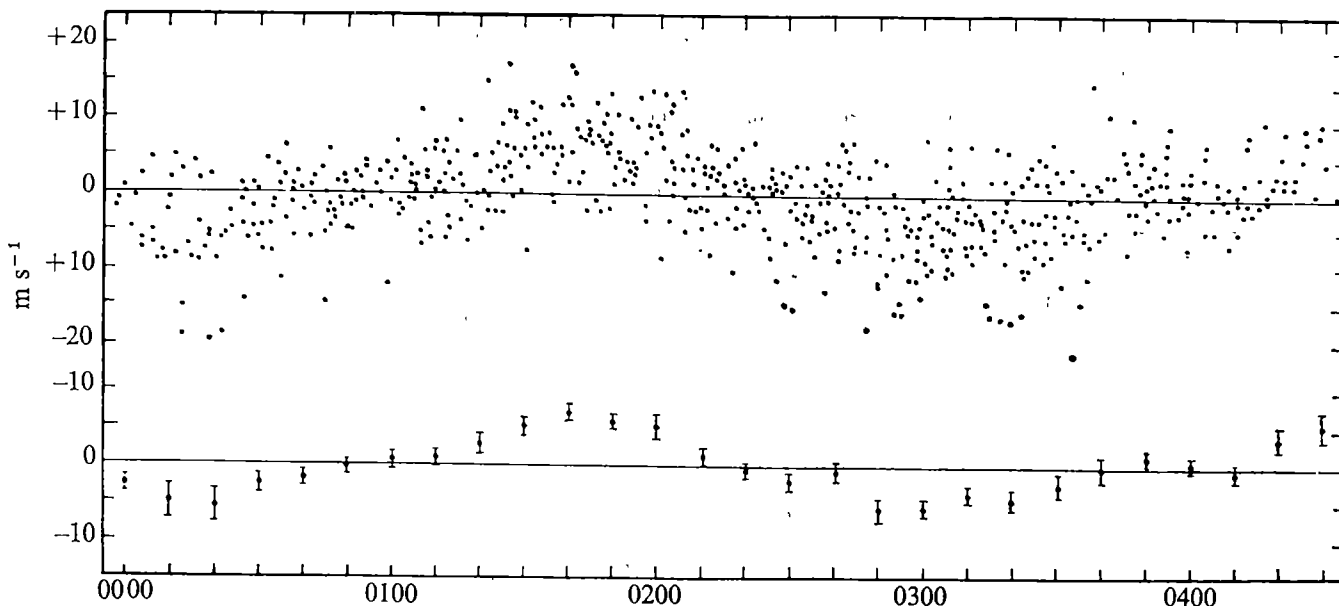
### Interpretation

It is tempting to speculate about the results obtained. The systematic appearance of stationary periodic fluctuations in radial velocities of large (comparable with the radius of the disk) portions of the solar surface points definitely to pulsations of the Sun as a whole, and not to the rather stochastic processes of the ascent and descent of gigantic supergranules. The period 2 h 40 min is too long to be ascribed to the envelope of the Sun (the region where gravity acceleration  $g \approx$  constant), where the period would be no longer than 40–60 min. It also

cannot be ascribed to a set of standing oscillations on the solar surface<sup>4</sup>, because the effect does not depend on the phase of solar rotation. It cannot be a travelling wave around the Sun because the velocity of such a wave would be too high,  $\approx 140$  km s $^{-1}$ .

The simplest interpretation is that we observed purely radial pulsations. The most striking fact is that the observed period 2 h 40 min is almost precisely the same as that following from the simple formula of Ritter for radial pulsations  $\sigma^2 = (3\gamma - 4)g/R_\odot$  giving  $P = 2\pi/\sigma = 2$  h 47 min for  $\gamma = 5/3$ , almost the value if the Sun were to be an homogeneous sphere. (It should also be noted here that for more inhomogeneous models, for example when  $\rho \sim r^{-2}$  or on Roche's model, we would get much shorter periods.) The question can be considered more accurately using the results of Chandrasekhar and Lebowitz<sup>5</sup>, or those of Cox *et al.*<sup>6</sup> for the oscillations of polytropic gaseous spheres. It is easy to find from these results the dependence of  $\sigma^2/4\pi G\rho_m\lambda$  on the increase in density  $\lambda = \rho_c/\rho_m$  towards the centre of the Sun, which shows that periods decrease with increase of  $\lambda$  and of polytropic index  $n$  (as well as with  $3\gamma - 4$ ) for all different modes of oscillations (pulsational, transverse

Fig. 2 The best fit for the fluctuations from visual inspection of the records obtained during first 9 d of observations. Each point is the 5-min integrated signal of radial velocity (top). Averaging this gives the sine-like curve shown below.



shear, and toroidal, modes). For all these, the periods seem to be too short to be compatible with observations unless the polytropic index is not higher than  $n \approx 0.5$ . If, for a moment, we assume such a value of  $n$ , it would lead us to  $\lambda \approx 1.5$ ,  $\rho_c \approx 2$  and  $T_c \approx 6.5 \times 10^6$  K, and, at the mean molecular weight  $\mu = 0.6$  ( $X = 0.73$ ,  $Y = 0.26$ ,  $Z = 0.014$ )<sup>7</sup>, we would get such a low rate of energy generation  $\varepsilon$  from proton-proton reactions (see Reeves<sup>8</sup>, formula (3.6)) that the luminosity

$$L = \int_0^{R_\odot} \varepsilon(p, p) 4\pi r^2 \rho dr$$

would be  $\approx 2.5 \times 10^{29}$  erg s<sup>-1</sup>,  $\sim 10^4$  times smaller than the observed value.

### Possible explanation

We have investigated two possible solutions to this dilemma. The first alternative is that nuclear, and, in particular, (p,p) reactions are not responsible for energy generation in the Sun. Such a conclusion, although rather extravagant, is quite consistent with the observed absence of appreciable neutrino flux from the Sun<sup>9</sup>, and with the observed abundance of Li and Be in the solar atmosphere. The second alternative pointed out to us by D. Gough, (personal communication) is to adopt the current model of solar structure with (p,p) reactions and assume that we really observe not pure radial pulsations but some gravity  $g$  mode of quadrupole oscillation. (The possibility of deformations of the type was also pointed out to us by Schatzman at our preliminary report on this work at the colloquium at Meudon observatory, May 21, 1975.)  $g$  modes ( $l = 2$ ) can yield long period oscillations<sup>10</sup>, and according to Gough's calculations (private communication) the period of the  $g_{11}$  mode is in perfect agreement with our observed period 2 h 40 min. It seems strange, however, that this high harmonic is dominant.

We should keep in mind that our present method does not allow us to distinguish pure radial pulsations from quadrupole oscillations. Neither does it allow us to observe dipole ( $l = 1$ ) oscillations at all. Our observations do, however, give some hint of small amplitude oscillations of a shorter period overlapped on the main 2 h 40 min oscillation. Spectral analysis of corresponding data will be given elsewhere.

We are inclined to believe that the problem arising here is much the same as in the case of Cepheids discussed long ago

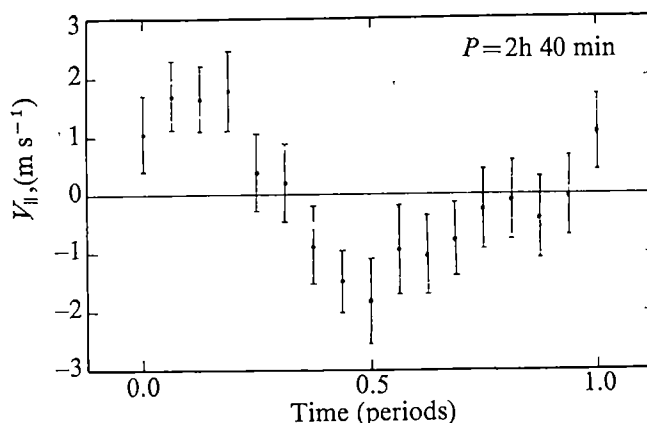


Fig. 3 Result of the superposed epoch analysis with 2 h 40 min period. Error bars represent r.m.s. deviations of individual measurements for each 10-min block of the data.

by Eddington<sup>11</sup> and Rosseland<sup>12</sup>, where the best agreement with observations can be reached for almost homogeneous spheres at  $\gamma = 5/3$ . We agree with Rosseland's conclusion that 'a thorough discussion of the problem on a new basis seems to be called for.'

We thank Dr D. Gough for helpful discussion of the paper before publication.

*Note added in proof:* Preliminary results of observations for 16 d in 1975 show the same periodicity in the mean magnetic field of the Sun, with an amplitude of 0.01 gauss.

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## Towards a heliological inverse problem

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*Theoretical periods of normal modes of vibration of the Sun are compared with the observed periods of oscillation of the solar surface. It is inferred from the comparison that it may soon be possible to use solar oscillations to measure aspects of the internal structure of the Sun.*

THE observational data that have been available for comparison with theoretical solar models are insufficient to enable stellar astrophysicists to ascertain the internal structure of the Sun. These data are just gross properties, such as the age, mass, radius, photon luminosity and neutrino luminosity, together

with details of the optical spectrum which are relevant only to the solar atmosphere. But recently there have been accurate measurements made of the frequencies of what are presumably normal modes of vibration of the Sun<sup>1-3</sup>. These dynamical data provide us with a new kind of information with which to probe the solar interior, and might lead to quite sensitive measures of certain aspects of the Sun's structure.

### Models of the Sun

Let us consider first how standard solar models are constructed. They are spherically symmetrical, and in hydrostatic and thermal balance. The assumption that the rotation rate in the

interior is comparable with that observed at the surface is usually made, which leads to negligible centrifugal forces and implies that spherical symmetry is a good approximation. This is consistent with the observed near circular shape of the solar image. It also leads to very low estimates of rotationally induced circulation currents. Thus, since solar models have convectively stable cores after the first few hundred Myr of their evolution on the main sequence, the products of thermonuclear reactions after that time are assumed not to have been transported away from the place where they were produced.

Models of stars of solar mass constructed from these assumptions predict a relationship between the photon luminosity  $L$  and time. This can easily be made consistent with the present solar luminosity  $L_{\odot}$  by adjusting the initial composition within the quite broad limits set by observation. Indeed, for any plausible initial abundance  $Z$  of heavy elements (usually assumed uniform) an initial hydrogen abundance  $X$  can always be found that reproduces  $L_{\odot}$ . The solar radius is not predicted because our ability to model the convection zone in the outer part of the star is inadequate. Instead convection theories are calibrated by adjusting solar models to have the correct radius at the appropriate age.

Thus, before the results of the attempt by Davis and his collaborators<sup>4,5</sup> to detect solar neutrinos were available, there was at least a single infinity of acceptable solar models, with varying  $Z$ , from which to choose. One of the roles of Davis' experiment was to help make the choice. It was not anticipated that the measured upper bound to the neutrino luminosity  $L_{\nu}$  would be a factor of  $\sim 10$  lower than the lowest theoretical predictions. Subsequently, models with low values of  $L_{\nu}$  were constructed<sup>6-13</sup>, though they did not satisfy all of the assumptions mentioned above. No doubt many more will emerge in the future. Some can be eliminated on theoretical grounds, such as those with hydrogen shells<sup>12</sup> or cores<sup>13</sup> which are Rayleigh-Taylor unstable, but the rest must be tested against new observations.

## Solar oscillations

An hypothesis that has received much attention in recent years is that the Sun has a rapidly rotating core. This has been pursued partly to produce models with low  $L_{\nu}$ , but principally to attempt to refute general relativity<sup>14</sup>. Careful observations of the oblateness of the quiet Sun by Hill and Stebbins<sup>15</sup> now makes the hypothesis unlikely. But these and other similar observations<sup>1,16</sup> have revealed that the apparent diameter of the solar image is oscillating, with a discrete spectrum of periods  $\lesssim 1$ h, and in the range of the low order  $p$  modes (acoustic modes). Severny, Kotov and Tsap<sup>2</sup> (in this issue of *Nature*) report observations of the difference between line of sight velocities at the poles and disk centre. This too is oscillatory, with a dominant period of 2 h 40 min, and is probably associated with a  $g$  mode (gravity wave). A spectrum of oscillations in a mean line-of-sight velocity has also been obtained recently by Brookes, Isaak and van der Raay<sup>3</sup> (also in this issue of *Nature*), who compared solar absorption lines with corresponding lines produced in the laboratory. The largest period observed is  $2.65 \pm 0.04$  h, in agreement with Severny *et al.* Fossat and Ricort<sup>36</sup> have recently reported large scale oscillations with a period of about 10 min.

Oscillations of the solar atmosphere have been observed before: the five-minute oscillations have been studied for more than a decade<sup>17</sup>, but these are probably a superposition of only those normal modes that have significant amplitudes in the extreme outer layers of the Sun (ref. 18, and H. Ando and Y. Osaki, unpublished), and so provide no direct information about the interior. Some 'long period oscillations' with periods of 40-50 min have also been detected<sup>19-22</sup>, but their periods were not accurately determined.

All the oscillations observed have too low an amplitude to influence the structure of the solar interior significantly, but their presence provides a new rich class of diagnostic data with which to compare the properties of theoretical models.

## Oscillations and solar structure

To what extent can the structure of the Sun be inferred from these comparisons? As a first step towards answering this question we have computed the low frequency ends of three spectra of  $p$  modes and the high frequency end of one spectrum of  $g$  modes. The  $p$  modes chosen have  $l = 0, 2$  and 4 and the  $g$  modes have  $l = 2$ , because these are the modes most likely to have been detected with the present techniques. The basic solar model was computed in the standard way with the program described by Eggleton<sup>23</sup>. With  $Z = 0.02$  and  $X = 0.735$  at zero age, and a mixing length of 1.10 pressure scale heights in Böhm-Vitense's<sup>24</sup> formulation of the mixing length theory, the model evolved to the present luminosity and radius in  $4.7 \times 10^9$  yr. The linearised oscillations were computed in adiabatic approximation in the manner described in ref. 25; non-adiabatic processes have a negligible influence on the frequencies of those modes with few nodes in the radial direction. The results are presented in Table 1, together with the frequencies of the peaks in the spectra reported by Hill, Stebbins and Brown<sup>16</sup>. To gain some idea of the sensitivity of the computed frequencies to variations in the basic solar model, the calculation was repeated with a heavy element abundance  $Z = 0.04$ . This required choosing  $X = 0.68$  at zero age and a mixing length of 1.325 pressure scale heights. The frequencies of a few of the modes are presented in Table 1 in parentheses. The frequencies in square brackets were obtained by changing the distribution of mesh points, and so indicate a lower bound to truncation errors.

Several features of Table 1 are noteworthy. First, it is clear that all but perhaps the lowest of the observed frequencies are approximately reproduced by the theory. This is evidence that the oscillations are not spurious and that most of them are indeed  $p$  modes. Not all the modes computed have been detected, however, though there is some evidence from the observed power spectrum displayed by Hill, Stebbins and Brown<sup>16</sup> for the existence of modes not listed in Table 1. For example, the  $p_4$  ( $l = 0$ ) and  $p_3$  ( $l = 2$ ) modes have frequencies near 0.63 mHz which is the position of a prominent shoulder in the power spectrum. Second, the frequencies of the radial ( $l = 0$ ) modes and the quadrupole ( $l = 2$ ) modes are very similar. Third, the effect on the  $p$  mode frequencies of changing  $Z$  is no greater than the observational uncertainties and the truncation errors in the calculations. The influence of composition changes on the  $g$  mode frequencies is somewhat greater, presumably because  $g$  modes are more concentrated towards the centre of the Sun, where the structure is more sensitive to the opacity. Indeed there is no  $g$  mode with  $l = 2$  (and none with  $l = 4$ ) with a period within the quoted errors of the observed period of 2 h  $40 \pm 1$  min when  $Z = 0.02$ , but there is when  $Z = 0.04$ . This cannot be considered as evidence for the higher  $Z$ , however, partly because the calculations are not sufficiently reliable, and partly because the identification of the mode may be incorrect: the oscillation may be a  $g_{10}$  ( $l = 2$ ) mode with  $Z \lesssim 0.02$ . (It is also evident that a very drastic change in the solar model would be necessary to enable the 2 h 40 min oscillation to be interpreted as the fundamental radial mode, as Severny *et al.*<sup>2</sup> and Brookes *et al.*<sup>3</sup> suggest. Indeed it is unlikely that any such model can be found that can generate the observed photon luminosity by thermonuclear reactions.)

## Future work

Although we are not yet in a position to draw definitive conclusions concerning the internal structure of the Sun from these observations, the results of this investigation do suggest that useful diagnostics from frequencies of oscillation will be available in the near future. To achieve this, greater accuracy must be attained from both the observations and the theory. Theorists have usually not considered it worthwhile to take the trouble to achieve high accuracy in the numerical integrations of the equations of stellar evolution, partly because our knowledge of opacities and nuclear reaction rates is uncertain and because



Table 1 Periods of solar oscillations (min)

1973	1975	$l = 0$		$l = 2$		$l = 4$		$l = 2$	
		Mode	Period	Mode	Period	Mode	Period	Mode	Period
52	47.9	$p_1$	62.7 (63.9)	$f$	46.0	$f$	39.6	$g_1$	55.1
		$p_2$	43.8	$p_1$	42.2 (42.3)			$g_2$	61.5
33	30.3	$p_3$	32.6 (31.8)	$p_2$	34.3	$p_1$	38.0	$g_3$	70.9
		$p_4$	26.0	$p_3$	26.7	$p_2$	29.3	$g_4$	81.8
23.8	21.0	$p_5$	21.0 [21.5]	$p_4$	21.5	$p_3$	23.4	$g_5$	93.0
		$p_6$	18.2	$p_5$	17.9	$p_4$	19.5	$g_6$	105
16.7	17.1	$p_7$	15.8 [15.4]	$p_6$	16.0	$p_5$	16.8	$g_7$	117
		$p_8$	14.0	$p_7$	14.1	$p_6$	14.6	$g_8$	130
13.3	11.8	$p_9$	12.5	$p_8$	12.6	$p_7$	13.0	$g_9$	142
		$p_{10}$	11.3	$p_9$	11.4	$p_8$	11.7	$g_{10}$	154 (147)
10.4	10.5	$p_{11}$	10.4	$p_{10}$	10.4			$g_{11}$	167 (159)
		$p_{12}$	9.55					$g_{12}$	180 (171)
9.2	8.8	$p_{13}$	8.84 [9.12]						
		$p_{14}$	8.52						
7.6	7.9	$p_{15}$	7.99						
		$p_{16}$	7.53						
7.0	7.2	$p_{17}$	7.12						
		$p_{18}$	6.75						
		$p_{19}$	6.41						
		$p_{20}$	6.10						

The periods headed 1973 and 1975 are from the observations reported by Hill, Stebbins and Brown<sup>16</sup>, and are accurate to  $\sim 5\%$ . Those periods not reported in the publication were kindly supplied by Professor Hill. Periods in the other columns were obtained theoretically using a solar model with  $Z = 0.02$ , except those in parentheses which are periods of oscillation of a model with  $Z = 0.04$ . The periods in square brackets were obtained by repeating the computation with  $Z = 0.02$  using a different distribution of mesh points. The classification of the modes, radial and non-radial, is according to the scheme proposed by Scuflaire<sup>34</sup> and Osaki<sup>35</sup>.

we cannot model thermal convection and other macroscopic motions reliably, and partly because there have been no observations that would distinguish between subtle differences in the models. But now the time has come to be a little more careful. Potential second-order accuracy difference schemes in some evolution programmes are spoiled, for example, by linearly interpolating opacity tables; this can quite easily be avoided. Accurate frequencies can be achieved by using variational principles on computed eigenfunctions. Also, more accurate observations are imminent. We do not know for how long a particular normal mode maintains an observable amplitude, but if it is many years, frequent observations over a long interval of time will yield very accurate periods. The observations by Severny *et al.*<sup>2</sup> and Brookes *et al.*<sup>3</sup> are a first step in this direction. Longer observing programmes are planned by Brown, Hill and Stebbins (personal communication) which may fix the  $p$  mode frequencies to within  $\frac{1}{4}\%$ , and make  $g$  mode periods measurable by their technique.

## Prospects

The prospect of such information is exciting, for it opens the possibility of a limited inverse problem of much the same kind as geophysicists pose to probe the internal structure of the Earth with seismic waves<sup>26,27</sup>. Because a spectrum contains so much information one can attempt to measure functions of radius  $r$  rather than just integral quantities such as luminosity. For example, given a certain set of assumptions such as hydrostatic support, thermal balance, an equation of state, opacities and nuclear reaction rates, one can seek that  $Z$  and that function  $X(r)$  that reproduces the observed spectra. If they do not

exist the assumptions must then be questioned: the answers may give a clue as to why standard solar models have been unable to yield neutrino fluxes consistent with Davis' observations.

To perform such an inversion, the modes exhibiting the observed frequencies must be correctly identified. It is apparent from Table 1 that it would be extremely difficult, if not impossible, to distinguish between  $p$  modes with  $l = 0$  and  $l = 2$  by frequency measurements alone, because the differences between their frequencies are less than variations that are produced by plausible changes in the composition of solar models. Instead,  $l$  must be measured directly, by observing the dependence of the amplitude and phase of the oscillations with position on the solar image. Hill and his collaborators plan to measure latitude dependence in the near future. This will yield several spectra, which in principle could be used to estimate simultaneously several functions such as  $X(r)$ . With enough spectra, a detailed model of the present Sun might be constructed without recourse to assumptions about the solar history. Of course since only finite portions of the infinite spectra can be measured, only limited resolution can be achieved, but with reasonable assumptions about smoothness one might hope to achieve considerably more reliable models than we have at present.

It is necessary to ask how many different oscillation spectra will be required to achieve this. The answer is not yet known, though Backus and Gilbert's<sup>27</sup> speculations on the geophysical inverse problem suggest that the number is somewhat greater (perhaps by almost a factor 2) than the number of functions to be measured. In any case, the reliability of the inferences increases with the number of spectra available.

It would thus be useful to add to the spectra that are currently being measured, the modes with  $l = 1$ . These modes are antisymmetrical about the Equator (assuming the axis of the mode coincides with the axis of rotation). The line-of-sight velocity varies with latitude  $\lambda$  as  $\sin 2\lambda$  and so vanishes at pole and Equator, but the technique described by Severny *et al.*<sup>2</sup> could presumably be modified to compare velocities at 45°N and 45°S. For Hill *et al.* to observe such a mode it would be necessary to observe the absolute position of the limb with respect to the other stars. This is more difficult than their present experiment, but may be possible in a year or so.

The driving of the oscillations must also be studied, for this can provide further diagnostic information just as the quality factors of seismic oscillations yield information about the dissipative properties of the Earth. Several studies of the growth or decay of normal modes of oscillation of the Sun have been reported<sup>25, 28–30</sup>, but the results are inconclusive. It is likely that the convection zone in the outer envelope is the seat of the excitation, because that is where the amplitudes of the oscillations are greatest. Spiegel<sup>31</sup> pointed out that the driving of non-radial  $p$  modes may take place in superadiabatic regions: this seems to be quite effective in the thin superadiabatic layer near the top of the zone for modes with high  $l$ , though the  $\kappa$ -mechanism, which drives the pulsations of Cepheids and RR Lyrae stars, is more important (H. Ando, and Y. Osaki, unpublished). It is likely that there is a superadiabatic region of much larger extent near the bottom of the convection zone, as is exhibited by models with mixing lengths that never exceed the distance from the nearest boundary of the convection zone<sup>32</sup> and is suggested by modal calculations of convection zones in A stars (J. Toomre, J.-P. Zahn, J. Latour, and E. A. Spiegel, unpublished). This may contribute towards driving modes with lower  $l$ . The direct influence of the convection itself is also likely to be important<sup>33</sup>, both in the way it modulates the heat flux and by the direct action of the Reynolds stresses. This presents a severe theoretical difficulty, as does the treatment of the reflection and transmission characteristics of the base of the corona. Indeed, these oscillations may be the principal source of energy maintaining the corona. It is important to inquire whether the continual interplay of these processes maintain the oscillations at approximately constant amplitude and phase, or whether, as some observations suggest<sup>20, 22, 36</sup>, the oscillations are triggered by occasional violent events such as flares, and then decay. If the former is the case, nonlinear pro-

cesses will determine the amplitudes; if the latter, it might be possible to approximate the decay using linear theory. In either case resonant interactions between modes may be important, and may explain why a particular mode is picked out from the closely spaced  $g$  mode spectrum. But whatever is occurring, the observed amplitudes will provide additional information that sooner or later will lead us to a better understanding of the Sun.

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# Observation of free oscillations of the Sun

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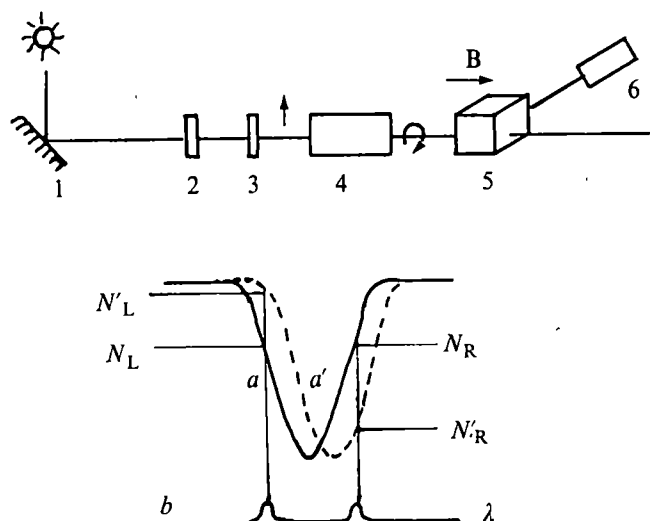
*The Fraunhofer absorption lines for potassium and sodium on the Sun are compared with the corresponding lines in the laboratory using a resonant optical scattering method. The observed shifts between the Sun and laboratory lines may be interpreted in terms of the gravitational redshift (GRS), motion of the laboratory relative to the Sun and oscillatory terms which may be related to oscillations of the Sun.*

IN 1961, one of us proposed a new method for solar observation<sup>1</sup>, which has since been applied successfully<sup>2–4</sup>. The method is best illustrated with reference to Fig. 1: A circularly polarised absorption line, represented by the solid upper curve *a*, is incident on a K or Na vapour placed in a longitudinal magnetic field of such a strength as to place the anomalous Zeeman components, curve *b*, near the steepest parts of the solar absorption line. The intensity of resonantly scattered

light of left-handed polarisation is given by  $N_L$  whereas that for right-handed polarisation is given by  $N_R$ . It is clear from the figure that when there is no relative displacement between the solar and laboratory lines,  $N_L$  equals  $N_R$ . If, however there is a relative shift between the two lines, as illustrated by the dotted upper curve *a'*, the two intensities are no longer equal. Since the narrow laboratory Zeeman components overlap the steepest parts of the broader solar line, this method is extremely sensitive to small shifts.

## Apparatus

The apparatus was developed in Birmingham during 1973, and taken to the observatory at the Pic-du-Midi in France during the autumn of 1974, where the present data were recorded. Unfortunately, of the 12 days on which observation was possible, only 2 yielded data of sufficient quality and duration to allow



**Fig. 1** Upper: Schematic view of apparatus (see text) Lower: Principle of apparatus showing solar absorption line *a* and the position of the normal Zeeman components *b*. The dotted curve *a'* is for a shifted line.

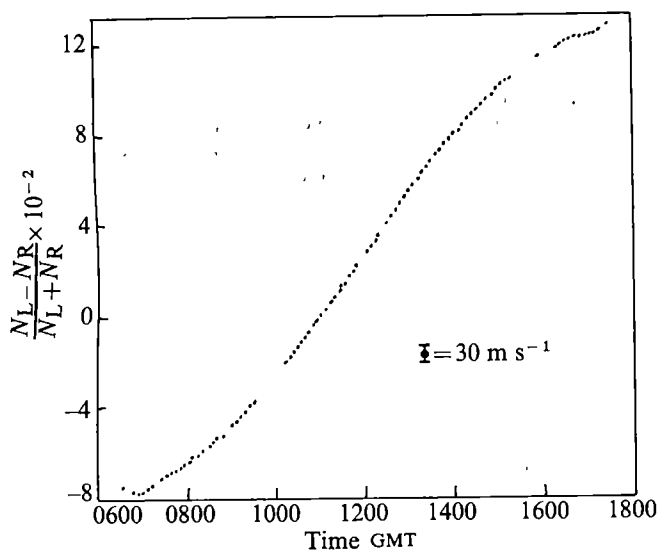
for analysis. A small equatorially mounted servo controlled heliostat (1) directs integral solar light, appropriately filtered by a thermostatically controlled interference filter, (2) through a polariser (3) and electro-optical light modulator (4) which produce the requisite left- or right-handed polarisation, by the application of the appropriate electric potential. The light thus enters a vapour cell (5) situated in the field of a permanent magnet; the resonantly scattered light is detected by a cooled photomultiplier (6), and the processed output pulses are recorded on digital magnetic tape for subsequent computer analysis.

Integral solar light was used to reduce random shifts from the motion of individual granules of the Sun, the five-minute oscillation, and differential shifts across the rotating solar disk. Displacements caused by imprecise guiding and atmospheric turbulence also become less serious.

## Results

The recorded data thus consist of numbers of counts  $N_L$  and  $N_R$ , taken at approximately 0.5-s intervals. The difference between these data points over their sum  $[(N_L - N_R)/(N_L + N_R)]$  then gives a measure of the line shift corrected for any intensity

**Fig. 2** Data obtained on September 27, 1974 using a K cell.



fluctuation. Each data point consisted of  $\sim 5 \times 10^5$  counts, and the mean of 20 such ratios were taken, rejecting any pair which showed a gross deviation from that mean. Thus data points were obtained at 20-s intervals with a statistical accuracy of  $\sim 2 \times 10^{-4}$  which corresponds to a shift of  $\sim 1 \text{ m s}^{-1}$ . Results obtained on September 27, 1974 are shown in Fig. 2.

## Analysis

The observed shift of the solar absorption line relative to the laboratory line may be expressed in terms of velocities as

$$V_{\text{obs}} = V_{\text{orbit}} + V_{\text{spin}} + V_{\text{GRS}} + V(t) \quad (1)$$

where

$$V_{\text{orbit}} = (V_{\odot} - V_E) \cdot \mathbf{r}$$

is the resolved component of the relative velocity of the Sun and Earth along the unit radius vector  $\mathbf{r}$  joining their centres, with due allowance for planetary and lunar perturbations<sup>6</sup>. The observer's velocity, at a time  $t$ , relative to the Earth's centre along  $\mathbf{r}$  is given by

$$V_{\text{spin}} = V_{\lambda} \cdot \mathbf{r} = \omega_E R_E(\lambda) \cos \lambda \cos \delta \sin \frac{\pi}{12} (t - t_0) \quad (2)$$

where  $\omega_E$  is the angular velocity of the Earth,  $R_E(\lambda)$  is the observer's distance from the centre of the Earth,  $\lambda$  the latitude,  $t_0$  is local noon and  $\delta$  is the declination of the Sun.

The gravitational redshift is

$$V_{\text{GRS}} = \frac{GM_{\odot}}{c} \left( \frac{1}{R_{\odot}} - \frac{1}{R_{\odot E}} \right) - \frac{GM_E}{cR_E}$$

+ (terms due to second-order Doppler shifts)

where  $M_{\odot}$ ,  $M_E$ ,  $R_{\odot}$  and  $R_E$  are the Sun and Earth masses and radii respectively, and  $R_{\odot E}$  is the distance from the Earth to the Sun. The gravitational constant is given by  $G$  and the velocity of light by  $c$ .

Finally,

$$V(t) = V_0 + V_1(t)$$

where  $V_0$  represents time-independent shifts due to, for instance, collision effects in the Sun, and  $V_1(t)$  contains time-dependent terms such as solar oscillations which form the basis for this report.

The terms  $V_{\text{orbit}}$  and  $V_{\text{GRS}}$  in equation (1) may be regarded as essentially constant during any one day, whereas the  $V_{\text{spin}}$  term varies sinusoidally as illustrated by equation (2). The amplitude of this term is  $\sim 340 \text{ m s}^{-1}$  during the autumn at Pic-du-Midi.

The data of Fig. 2 clearly indicate the sinusoidal variation and provide an accurate calibration of the apparatus in terms of known parameters. It is further clear that the data pass through zero, indicating that at this time the solar absorption line and the laboratory line have a zero relative shift. Thus

$$-V_{\text{GRS}} = V_{\text{orbit}} + V_{\text{spin}} + V(t)$$

If it is assumed that  $V_1(t)$  terms are small or may be averaged out, then the gravitational redshift may be determined directly from this 'null' experiment in terms of known parameters. Details of this measurement will be reported elsewhere.

To investigate the  $V_1(t)$  terms, a two-parameter fit of the form

$$a + b \sin \frac{\pi}{12} (t - t_0)$$

was made to the data. The residuals, representing the differences between this fitted curve and the actual data points, were then found and are shown in Fig. 3 for the data obtained on September 27 using a K vapour cell. These data clearly suggest an

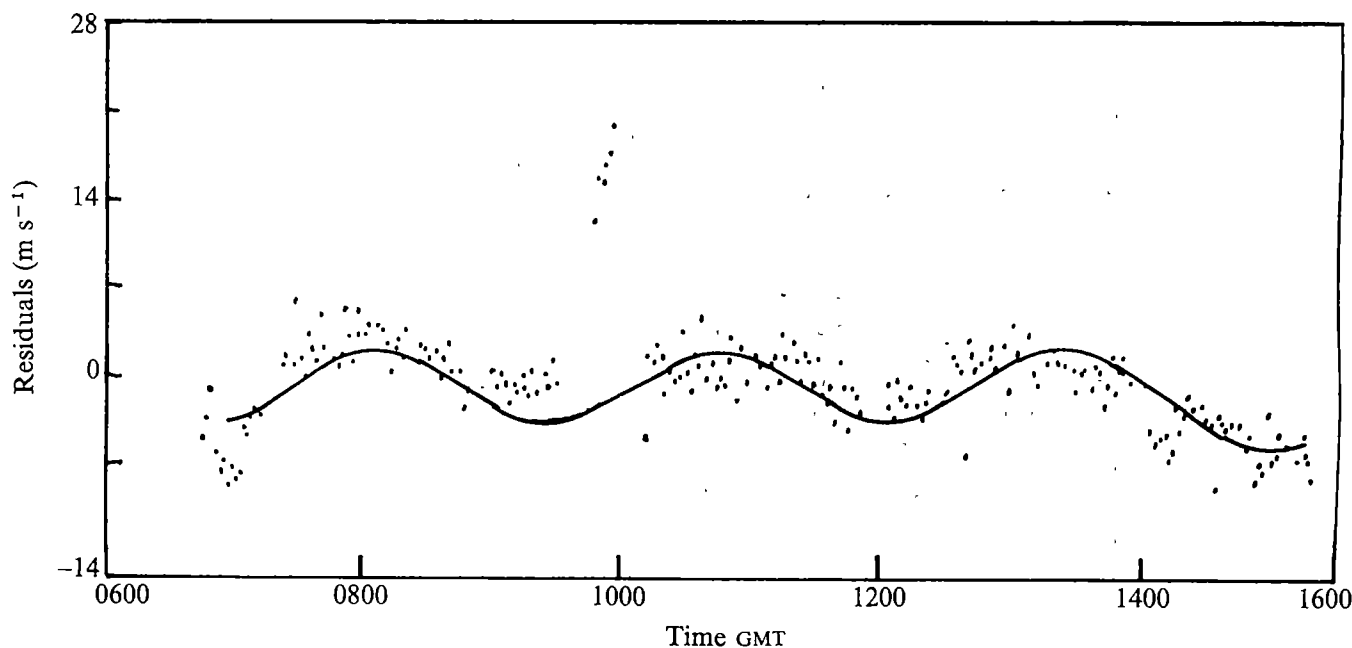


Fig. 3 Plot of residuals showing fitted 2.65-h oscillation.

oscillatory form for  $V_1(t)$  with a period between 2 and 3 h. A least squares fit to these points yielded a sine wave of amplitude  $2.70 \pm 0.24 \text{ m s}^{-1}$  and a period of  $2.65 \pm 0.04 \text{ h}$ . Similar data, but of lower quality, obtained on November 2 using a Na vapour cell gave an amplitude of  $4.5 \pm 0.7 \text{ m s}^{-1}$  for an oscillation of period  $2.7 \pm 0.1 \text{ h}$ .

The data of September 27 were further subjected to an autocorrelation and power spectrum analysis using a Hamming window. The resultant power spectrum, see Fig. 4, demonstrates clearly the existence of the 2.65-h period and suggests the presence of two or three further peaks whose amplitudes and periods are given in Table 1.

### Interpretation

The observed oscillations could be due to oscillations of the Sun or may be attributed to other effects. These other effects include the terrestrial atmosphere, nonlinearities in the apparatus, poor servo guiding, local voltage fluctuations, temperature instabilities and several more detailed instrumental effects. These causes, however, have been rejected after careful consideration of the types and magnitudes of variation that they might produce.

It is thus suggested that the 2.65-h period be identified with the gravest radial mode of oscillation of the Sun, the 58-min period with the fundamental non-radial mode and the 40-min

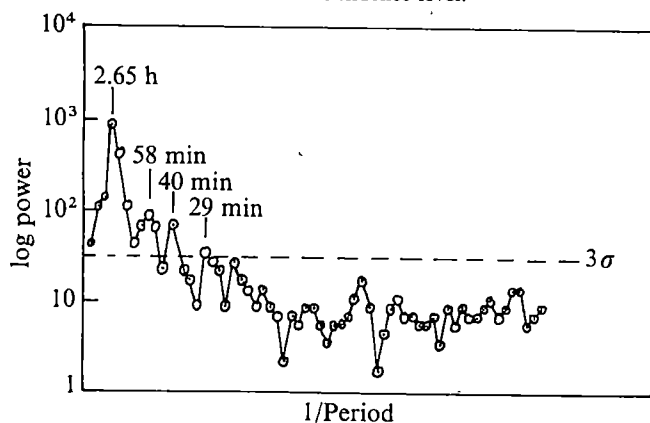
period with the second harmonic of the radial mode. The remaining peak in the power spectrum at 29 min can be traced to instrumental effects associated with the heliostat drive.

Extrapolation of the period-density relation of various pulsating stars<sup>8</sup>, if taken as a rough guide, places the expected period ( $P_0$ ) for the Sun  $1 \text{ h} < P_0 < 4 \text{ h}$ . Theoretical models of pulsations<sup>7-9</sup> indicate a similar, but narrower, range for the gravest radial mode, with the fundamental period  $P_0$  decreasing from 2.78 h to  $< 1 \text{ h}$  as the central density of the Sun increases. Current solar models<sup>9</sup> predict a period of  $\sim 1 \text{ h}$  corresponding to a steep density increase in the solar interior, in marked contrast to the observed 2.65-h period, which is consistent with a nearly homogeneous model of the Sun.

Table 1 Power spectrum of observed observations

Period	Observed amplitude ( $\text{m s}^{-1}$ )	Period predicted by homogeneous model
2.65 h	2.7	2.78 h: radial, fundamental
58 min	0.8	59 min: non-radial, fundamental
40 min	0.7	47 min: radial, second harmonic or 42 min: non-radial, second harmonic (instrumental)
29 min	0.5	

Fig. 4 Logarithmic power spectrum showing the four peaks above the  $3\sigma$  confidence level.



The value of  $P_0$  depends on the local velocity of sound,  $(\gamma kT/m)^{1/2}$ , integrated over the Sun, and if  $\gamma$  and  $m$  are presumed to be known,  $P_0$  becomes a measure of  $T$ , the mean temperature. The present value of  $P_0$ , assuming  $\gamma = 5/3$  and the usual composition for the Sun, yields a lower value for  $T$ , which in turn would produce a lower neutrino flux<sup>10,11</sup>.

If indeed the 58-min line is the fundamental of a non-radial mode of oscillation, then this line should be split by the rotation of the Sun<sup>12</sup>. Since the degree of splitting increases with the speed of rotation of the interior and since there is no evident splitting of the observed 58-min line, it may be concluded that the interior of the Sun rotates more slowly than once every 12 h.

The peak in the power spectrum corresponding to a 40-min period, is identified with the second harmonic of the radial mode. Oscillations of this period have been previously detected<sup>13</sup> and were also found with our apparatus during short bursts of good visibility at Birmingham during the spring of 1974.

The somewhat higher amplitude of the oscillations of November 2 for Na atoms, compared with that for K atoms on



September 27, is semi-quantitatively in agreement with expectations for a steady oscillation over this time interval. Sodium atoms in their ground state, being more abundant than potassium atoms, provide unit optical depth at a lower density in the solar photosphere, at which an unattenuated sound wave would have a higher velocity amplitude.

Each observation, taken separately, suggests that the damping is small ( $Q > 10$ ). If it is assumed that the oscillation observed on November 2 is a continuation of that first seen on September 27, and that no substantial excitation occurred during the intervening time, then  $Q$  becomes  $> 300$ , consistent with theoretical expectations<sup>14</sup>. The total energy content for the fundamental mode of oscillation integrated over the entire Sun is  $\sim 10^{31}$  erg, comparable with the energy of a major solar flare. In view, of the large  $Q$  for this mode of oscillation, however, the actual energy dissipated is negligible by solar standards.

The data could also be explained in terms of the high order  $g$  modes of non-radial oscillation<sup>15</sup>. These are predominantly horizontal and can have very long periods. It is difficult, however, to understand why other high order modes have not been detected unless some preferential excitation mechanism exists.

Hill<sup>16</sup> has reported the detection of solar oscillations during his measurements on solar oblateness. The periods reported are all  $< 1$  h and the amplitudes at least an order of magnitude greater than those reported here. It seems difficult to reconcile these data with our work, unless the oscillations are of high

order, so that averaging over the whole solar disk reduces their velocity amplitude substantially.

Improvements in the measurements may help to cast some light on the internal rotation of the Sun and thereby on the Einstein and Dicke theories of gravitation<sup>17</sup>. An extension of the work using a two-dimensional spectrometer capable of measuring wavelength shifts across the solar disk may well inaugurate a new field—the probing of the Sun's interior with seismic waves, analogous to its terrestrial counterpart<sup>18</sup>.

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## Presence of gene for $\beta$ globin in homozygous $\beta_0$ thalassaemia

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*In one southern Italian and one Pakistani patient with homozygous  $\beta_0$  thalassaemia in which no detectable  $\beta$ -globin synthesis occurs and no  $\beta$ -globin messenger RNA is found, the gene for  $\beta$  globin has been shown to be present using complementary DNA. This demonstrates that for these patients the imbalance in chain synthesis is not attributable to a gene deletion.*

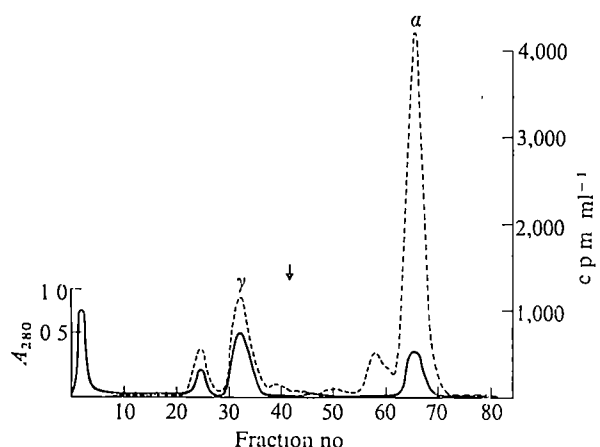
THALASSAEMIA is a common hereditary anaemia which is caused by imbalanced synthesis of  $\alpha$ - and  $\beta$ -globin chains<sup>1</sup>. In  $\alpha$  thalassaemia there is reduced synthesis of  $\alpha$ -globin, which in at least some cases results from a partial or complete deletion of the  $\alpha$ -globin genes<sup>2–4</sup>. Other cases of  $\alpha$  thalassaemia are caused by chain termination mutants for which the globin chain is synthesised in very small amounts<sup>5</sup>.

$\beta$ -Thalassaemia is a more complex hereditary condition, since the decrease in  $\beta$ -globin synthesis can be compensated by increased synthesis of  $\delta$  and  $\gamma$ -globin chains (as in  $\beta_0$  thalassaemia) or  $\gamma$ -globin chains only (as in  $\delta\beta_0$  thalassaemia). In cases

of  $\beta^+$  thalassaemia low levels of  $\beta$ -globin synthesis are observed, whereas in  $\beta_0$  thalassaemia no  $\beta$ -chain synthesis occurs and only haemoglobins  $A_2$  (HbA<sub>2</sub>) and F (HbF) are found in the peripheral blood. In some cases of  $\beta_0$  thalassaemia, messenger RNA (mRNA) for  $\beta$  globin is absent as judged by hybridisation to complementary DNA (cDNA) prepared using viral reverse transcriptase<sup>6</sup> and also by translation in cell-free protein synthesis systems<sup>7</sup>. In one form of  $\beta_0$  thalassaemia, from the Ferrara region of Italy,  $\beta$ -globin mRNA (mRNA <sub>$\beta$</sub> ) is present in erythroid cell cytoplasm but is not translated<sup>8</sup>.

We have isolated DNA from spleens of two patients with homozygous  $\beta_0$  thalassaemia. In neither case was there any demonstrable synthesis of  $\beta$  globin in the peripheral blood, nor could mRNA <sub>$\beta$</sub>  be detected in the cytoplasmic RNA of spleens or peripheral blood cells. In both cases, however, the hybridisation of cellular DNA to a DNA enriched in sequences specific for  $\beta$ -globin genes (cDNA <sub>$\beta$</sub> ) was identical to that of normal DNA, demonstrating the presence of substantially intact genes for  $\beta$  globin in homozygous  $\beta_0$ -thalassaemic patients.

This confirms our previous report of the presence of  $\beta$ -globin genes in the genetically more complex double heterozygote for



**Fig. 1** Globin synthesis in homozygous  $\beta_0$  thalassaemia. Washed red blood cells from the Pakistani patient with homozygous  $\beta_0$  thalassaemia were incubated in the presence of  $^3\text{H}$ -leucine (specific activity 40–60 Ci mmol $^{-1}$ , Radiochemical Centre) as described previously<sup>15</sup>. Extracted whole-cell globin was chromatographed on carboxymethylcellulose<sup>10</sup>. Aliquots of the column fractions were monitored for absorption at 280 nm and for radioactivity. Essentially no radioactivity (-----) associated with absorbance (—) was seen in the  $\beta$ -globin region (arrow:  $\beta$ -globin peak fraction from parallel marker run). Identical results were obtained with red blood cells from the Italian patient.

$\beta_0/\delta\beta_0$  thalassaemia, for which at least one of the haploid genomes was shown to contain a largely intact  $\beta$ -globin gene<sup>9</sup>. It remains to be determined whether the small deficit in  $\beta$ -gene hybridisation shown for that genome is due to a partial deletion in the haploid  $\delta\beta_0$ -globin gene region.

Spleens were removed for therapeutic reasons from two patients with  $\beta_0$  thalassaemia, for both of whom genetic data confirmed the haematological diagnosis. One patient was of southern Italian origin and the other from Lalipur, Pakistan. In both cases incubation of peripheral blood with  $^3\text{H}$ -leucine followed by globincha in separation on carboxymethylcellulose columns in 8 M urea-mercaptoethanol-phosphate buffer systems, pH 6.7 (ref. 10) showed  $\alpha$ - and  $\gamma$ -globin chain synthesis only. No  $\beta$ -globin synthesis was observed (Fig. 1, Pakistani patient; identical data were obtained from the Italian patient). In both cases, both parents showed elevated levels of HbA<sub>2</sub>.

Nuclear DNA was prepared from spleen using hydroxylapatite-urea as described previously<sup>2,11</sup>. Normal DNA was prepared either from non-thalassaemic spleen or from normal placenta. The DNA was fragmented to approximately 200–300 nucleotides in length either by sonication as described previously<sup>11</sup> or by boiling for 20 min in 0.3 N NaOH<sup>12</sup>.

Normal adult human globin mRNA (mRNA $^{\alpha,\beta}$ ) was prepared from reticulocyte-rich blood from a patient with a non-thalassaemic haemolytic anaemia. Cord exchange transfusion blood was obtained from newborn infants with Rhesus incompatibility, and used to prepare mRNA $^{\alpha,\gamma,\beta}$ .  $\alpha$ -Thalassaemia globin mRNA (mRNA $_{\text{HbH}}$ ) was obtained from blood from a patient with haemoglobin H disease<sup>2,9,13</sup>. Total  $\beta_0$ -thalassaemia cytoplasmic RNA (RNA $_{\beta_0\text{thal}}$ ) was prepared by phenol-chloroform extraction from  $\beta_0$ -thalassaemic spleen postnuclear supernatant<sup>9</sup>.

cDNA was prepared from the various mRNAs as template, using oligo(T) as primer with avian myeloblastosis virus reverse transcriptase as described previously<sup>14</sup>. The labelled nucleoside triphosphate used was  $^3\text{H}$ -dCTP (specific activity 25–27 Ci mmol $^{-1}$ ; Radiochemical Centre).

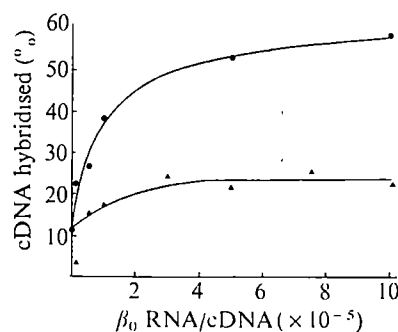
All cDNAs prepared from mRNAs hybridised back to their respective templates showing approximately 5–10% apparent double-stranded material in the absence of RNA and 10–15% material which did not hybridise even in great mRNA excess.

Complementary DNA enriched in sequences complementary to mRNA $_{\beta}$  was prepared by annealing cDNA $_{\text{HbH}}$ , which is already rich in cDNA $_{\beta}$ , to the cytoplasmic mRNA from the

previously described case of  $\delta\beta_0/\beta_0$  thalassaemia, which contains no detectable mRNA $_{\beta}$  sequences. The non-hybridising sequences (cDNA $_{\beta}$ ) do not bind to hydroxylapatite in 0.12 M sodium phosphate buffer, and were collected, concentrated and characterised as described previously<sup>9</sup>.

Three cDNA $_{\beta}$  probes were used in the experiments described below. cDNA $_{\text{HbH}}$  was approximately 80% cDNA $_{\beta}$  sequences. cDNA $_{\beta}$  cycled through hydroxylapatite as described above was >80% pure in one case and >90% in the other, as judged by hybridisation to  $\delta\beta_0/\beta_0$ -thalassaemic total cytoplasmic RNA<sup>9</sup>. Both cDNA $_{\beta}$  preparations showed plateau levels of approximately 70% when hybridised to a large excess of mRNA which contained  $\beta$ -globin. This reduction in plateau level was due to enrichment of non-hybridising material during hydroxylapatite purification of these cDNA $_{\beta}$  (ref. 9).

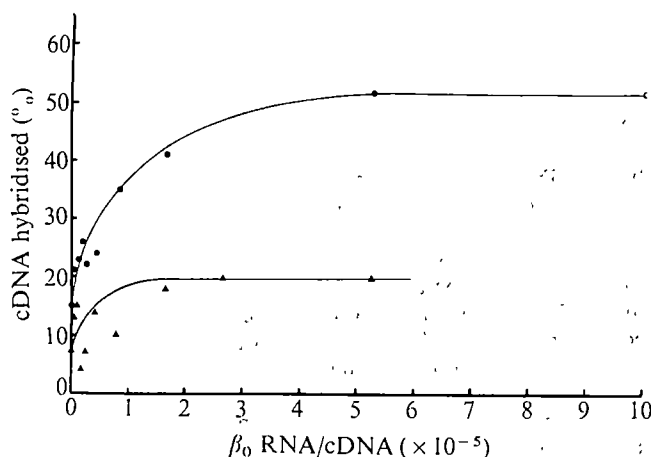
No  $\beta$ -globin mRNA was detected in the spleen cytoplasmic RNA from each patient (Figs 2 and 3). At a  $1 \times 10^6$ -fold



**Fig. 2** Hybridisation of  $\beta_0$  spleen (Pakistani patient) total cytoplasmic RNA with cDNA $_{\alpha,\beta,\gamma}$  and cDNA $_{\beta}$ . Total cytoplasmic RNA from  $\beta_0$  spleen was prepared from the post-nuclear supernatant of spleen homogenate by phenol-chloroform extraction<sup>9</sup>. mRNA was prepared by affinity chromatography with oligo(dT)-cellulose (Collaborative Research) from total RNA prepared by phenol-chloroform extraction of foetal, and HbH disease, whole red blood cells. DNAs were prepared to these mRNA templates, using RNA-dependent DNA polymerase from avian myeloblastosis virus (AMV), as described previously<sup>9,14</sup>.  $^3\text{H}$ -dCTP (Radiochemical Centre) of specific activity 27 Ci mmol $^{-1}$  was the only labelled nucleotide present. Specific activity of the cDNAs was 16,100 c.p.m. ng $^{-1}$  at a counting efficiency of 38%. cDNA $_{\text{HbH}}$  was used to prepare cDNA $_{\beta}$ , by hybridisation to a 10,000-fold excess of  $\delta\beta_0/\beta_0$  total cytoplasmic RNA, followed by isolation of the unhybridised, single-stranded fraction by chromatography on hydroxylapatite<sup>9</sup>. Purity of cDNA $_{\beta}$  was estimated to be >80% (see text). A constant amount of 0.05 ng cDNA $_{\alpha,\beta,\gamma}$  or cDNA $_{\beta}$  was hybridised with increasing amounts of  $\beta_0$ -spleen cytoplasmic RNA, in a volume of 2  $\mu\text{l}$  50% formamide, 0.5 M NaCl, 25 mM HEPES buffer, pH 6.8, 1 mM EDTA, containing 2.5 mg ml $^{-1}$  *Escherichia coli* RNA. Samples were sealed in silicone-coated glass capillaries, and incubated at 43°C for 168 h. Percentage of cDNA which had hybridised was determined by assay with the single-stranded-specific nuclease, S1, followed by perchloric acid precipitation of hybridised material. Hybridisation of cDNA $_{\alpha,\beta,\gamma}$  (●) and cDNA $_{\beta}$  (▲) to increasing amounts of  $\beta_0$ -spleen cytoplasmic RNA is shown. Both cDNAs contain a double-stranded component of approximately 11%. Hybridisation of both cDNAs, in the same conditions, to a  $1 \times 10^6$ -fold excess of *E. coli* RNA, still gave values of 11% for the double-stranded component.

excess of  $\beta_0$ -cytoplasmic RNA, cDNA $_{\beta}$  and cDNA $_{\text{HbH}}$  hybridised to approximately 12% above background, indicating a virtual absence of  $\beta$ -globin sequences. The low level of hybridisation of the  $\beta$ -enriched cDNAs is attributable to contaminating  $\alpha$ - or  $\gamma$ -globin sequences in the cDNA hybridising to their complementary sequences in the  $\beta_0$ -cytoplasmic RNA.

In contrast,  $\alpha$ - and  $\gamma$ -globin sequences were readily detected by the cDNA $_{\alpha,\beta,\gamma}$  (Fig. 2) and cDNA $_{\alpha,\beta}$  (Fig. 3) in both preparations of spleen  $\beta_0$ -cytoplasmic RNA at RNA-cDNA ratios as low as  $1 \times 10^5$ . The fact that cDNA $_{\alpha,\beta}$  and cDNA $_{\alpha,\beta,\gamma}$



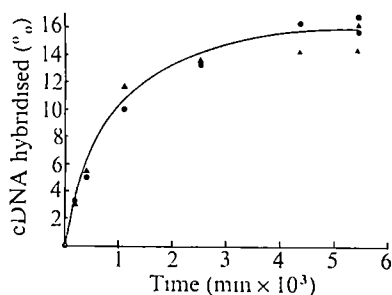
**Fig. 3** Hybridisation of  $\beta_0$ -thalassaemic spleen (Italian patient) total cytoplasmic RNA with  $cDNA_{HbH}$  and  $cDNA_{\alpha, \beta}$ .  $\beta_0$ -spleen cytoplasmic RNA,  $cDNA_{HbH}$  and  $cDNA_{\alpha, \beta}$  were prepared as described in Fig. 2.  $cDNA_{HbH}$  was estimated to contain 80%  $\beta$ -globin sequences<sup>9</sup>. A constant amount (20 pg) of  $cDNA_{HbH}$  ( $\blacktriangle$ ) or  $cDNA_{\alpha, \beta}$  ( $\bullet$ ) was hybridised with increasing amounts of  $\beta_0$ -spleen cytoplasmic RNA in 1  $\mu$ l hybridisation buffer at 43 °C for 10 d. Proportion of cDNA rendered resistant to S1 nuclease digestion was determined. Both  $cDNA_{HbH}$  and  $cDNA_{\alpha, \beta}$  hybridised to 80–85% to their respective templates.

do not hybridise to levels greater than 50–60%, however, is further evidence for the absence of  $\beta$ -globin sequences.

Hybridisation to total normal and  $\beta_0$ -thalassaemic DNA was carried out in  $cDNA_{\beta}$  excess. Figure 4 shows the rate of reannealing of the  $cDNA_{HbH}$  to the DNA from the Pakistani patient. The curves for normal and  $\beta_0$ -thalassaemic DNA are essentially identical.

To demonstrate with greater rigour the equivalence of the thalassaemic and normal DNAs, the extents of hybridisation were determined for, both cases at a number of different  $cDNA$ -DNA ratios. At low ratios of  $cDNA_{\beta}$ -total DNA, the effects of contaminating  $\alpha$ - and  $\gamma$ -globin  $cDNA$  sequences will be greatly reduced. At ratios varying from 6 pg  $cDNA_{\beta}$  to 100  $\mu$ g total DNA to 70 pg  $cDNA_{\beta}$  to 100  $\mu$ g total

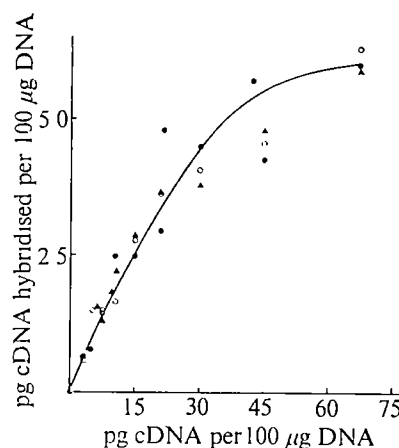
**Fig. 4** Hybridisation of excess  $cDNA_{\beta}$  to normal and  $\beta_0$  DNA (Pakistani patient) DNA was prepared from normal and  $\beta_0$  spleen, and fragmented to a mean size of 200–300 nucleotides single-stranded length, by sonication, as described previously<sup>8,11</sup>. Samples of 2.16 mg normal, or  $\beta_0$  DNA, and 0.5 ng  $cDNA_{\beta}$  were lyophilised and dissolved in 0.12 M sodium phosphate buffer, pH 6.8, at a DNA concentration of 5 mg ml<sup>-1</sup>. Aliquots (43  $\mu$ l) (one-tenth total volume) were sealed in silicone-treated glass capillaries, boiled for 10 min, and incubated for various times at 60 °C. At selected times, contents of capillaries were expelled with 210  $\mu$ l S1 nuclease assay buffer and stored at -20 °C until assayed with S1 nuclease to determine the proportion of  $cDNA$  which had hybridised<sup>9</sup>. A background S1 resistance of approximately 2% has been subtracted, and percentage hybridisation has been calculated relative to the maximum amount of hybridisable  $cDNA_{\beta}$  (70%).  $\bullet$ ; Percentage  $cDNA_{\beta}$  hybridised to normal DNA;  $\blacktriangle$ , percentage  $cDNA_{\beta}$  hybridised to  $\beta_0$  DNA.



DNA the extent of hybridisation is the same for the normal and the two thalassaemic DNAs (Fig. 5).

Analyses of both thalassaemic DNAs with  $cDNA_{\alpha, \gamma, \delta}$  in excess demonstrated a complexity of the total globin genes identical to that of normal DNA<sup>9</sup>.

We have previously demonstrated cross hybridisation between  $\beta$ -globin and  $\delta$ -globin gene sequences<sup>9</sup>. It is known from genetic<sup>1</sup> and molecular<sup>9</sup> analyses that there are single copies of each of these genes, and a deletion of the  $\beta$ -globin gene would be apparent as a 50% reduction in extent of hybridisation and greater than 50% reduction (because of mismatching) in rate of hybridisation. The identity of the hybridisation for normal and thalassaemic DNAs demonstrates that no substantial deletion of the  $\beta$ -globin gene has occurred.



**Fig. 5** Hybridisation of  $cDNA_{\beta}$  to normal and  $\beta_0$  DNAs as a function of  $cDNA$ -DNA ratio. Normal or  $\beta_0$ -thalassaemic DNA was prepared from spleens as described in Fig. 3, except that fragmentation to 200–300 nucleotides was achieved by boiling in 0.3 N NaOH for 20 min.  $cDNA_{\beta}$  (>90% pure) was mixed at the indicated ratios with normal or thalassaemic DNA (50–250  $\mu$ g) in 0.12 M sodium phosphate buffer, pH 6.8, 1 mM EDTA, at a DNA concentration of 5 mg ml<sup>-1</sup>. Mixtures were sealed in capillaries, heated to 100 °C for 10 min, and incubated at 68 °C for 5,200 min. Samples were diluted with S1 nuclease assay buffer (500  $\mu$ l per 100  $\mu$ g DNA) and assayed with S1 nuclease to measure the proportion of  $cDNA$  hybridised<sup>9</sup>. A background S1 resistance of 2% has been subtracted, and the percentage of  $cDNA_{\beta}$  hybridised has been calculated relative to the maximum amount of hybridisable  $cDNA_{\beta}$  (70%). Results are shown as pg  $cDNA_{\beta}$  hybridised per 100  $\mu$ g DNA as a function of  $cDNA$ -DNA ratio.  $\bullet$ , Normal DNA;  $\circ$ ,  $\beta_0$ -thalassaemic DNA (Italian patient),  $\blacktriangle$ ,  $\beta_0$ -thalassaemic DNA (Pakistani patient).

Since the  $cDNA_{\beta}$  used in these experiments is not a complete copy of the mRNA <sub>$\beta$</sub> , a deletion near to the initiation region of transcription, to the 5' end of the mRNA, would be more difficult to detect. A deletion in a non-transcribed portion of the genome which might still be involved in the control of transcription could not be detected using these techniques.

$\beta_0$  thalassaemias from other areas (for example, Ferrara<sup>8</sup> and of Chinese origin<sup>10</sup>) contain globin mRNA sequences which are untranslated. This result for homozygous  $\beta_0$  thalassaemia from two separate geographical locations is in marked contrast to the previous demonstration that a gene deletion is the primary cause of south-east Asian homozygous  $\alpha_0$  thalassaemia. It is still not clear whether transcription of the  $\beta$ -globin RNA sequence is completely absent, either because of a mutation in the initiation region for transcription or a mutation of a necessary control element, or if transcription occurs but the mRNA <sub>$\beta$</sub>  sequences are either not processed from nucleus to cytoplasm or are broken down very rapidly without translation.

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# letters to nature

## Transient X-ray source A1118—61

THE transient X-ray source Ariel 1118—61 was discovered late in 1974 (refs 1, 2). It has been suggested<sup>3</sup> that this source may be associated with the long period Mira-type variable RS Cen, which is located within the error box for the X-ray source. The proposed mechanism of X-ray generation was accretion on to a compact object in orbit around the variable star. The periodic variation in the radius of the star would lead to a variable accretion rate at the orbit of the compact object, and give rise to a variable X-ray flux. This suggestion has two important consequences. First, it raises the possibility that some, at least, of the apparently transient X-ray sources can be explained by similar phenomena. For instance, Barnden and Francey<sup>4</sup> and Shukla and Wilson<sup>5</sup> reported the presence of two presumably 'transient' X-ray sources in the constellation Cetus. The error box for each source (Cetus X-1 (ref. 4) and Cetus X-2 (ref. 5)) was large,  $\sim 10$ – $15^\circ$  diameter, but they did just overlap. Of significance here is the fact that the overlap area is centred on O Ceti (Mira), the type star of this class of variables. The two observations were at similar phases (0.12 and 0.22) of the variable, but separated in time by  $\sim 1$  cycle. The difference in phase of the Centaurus and Cetus transients is easily explained by differences in orbit size and expansion velocity.

The second consequence of Fabian *et al.*'s proposed identification is a clear, experimentally testable prediction, that the X-ray source should be observable at intervals of  $\sim 164.5$  d after the outburst observed by Ariel V.

We have used the collimated proportional counter on board Copernicus to test this prediction. The detector was pointed at A1118—61 on June 8, 1975 between 17 h 9 min and 22 h 15 min, a time corresponding to an eclipse of the nearby binary X-ray source Cen X-3. No signal was detected above background during the whole of the observing session. Our 90% confidence upper limit to the source strength in the energy band 2.5–7.5 keV is  $3 \times 10^{-11}$  erg cm<sup>-2</sup> s<sup>-1</sup>; this is 1.7% of the peak flux reported by Ariel V in the same energy band. Thus it is likely that the X-ray source A1118—61 is not connected with RS Cen, unless for some reason the X-ray generation was turned off during the time when Copernicus was observing the source.

Copernicus observed Cen X-3 on January 30, 1974 precisely 2 periods of RS Cen before the peak of the outburst reported by Ariel V. A1118—61 was well within the field of view of the detector, so we have been able to search for a signal from it during the binary down state of Cen X-3. Again, no signal was detected above an upper

limit of  $2 \times 10^{-10}$  erg cm<sup>-2</sup> s<sup>-1</sup> ( $\sim 10\%$  of peak Ariel V flux) in the energy band 2.5–7.5 keV. Thus there is no strong case for an association of A1118—61 with RS Cen, based on the mechanism suggested by Fabian *et al.*<sup>3</sup>

We have also searched for X rays from A1118—61, using Copernicus, at a time well removed from the hypothesised critical phase. The observations were made between April 1975 6 d 23 h 31 min and 7 d 3 h 33 min, again during a binary down state of Cen X-3. On this occasion, too, we were unable to detect the source. Our 90% confidence upper limit on this occasion of  $\sim 9 \times 10^{-11}$  erg cm<sup>-2</sup> s<sup>-1</sup> in the energy band 2.5–7.5 keV is again substantially below the peak Ariel V flux of  $1.8 \times 10^{-9}$  erg cm<sup>-2</sup> s<sup>-1</sup>.

Our upper limits should clearly be borne in mind in any attempts to explain this source. In particular, Pacini and Shapiro<sup>6</sup> have suggested that it is the motion of the compact object in an eccentric orbit which periodically brings the compact object close enough to the primary for accretion rates to become appreciable (this scheme has also been suggested by Clark *et al.*<sup>7</sup> and Davison and Tuohy<sup>8</sup> to explain the long term behaviour of Cir X-1). Our upper limits modify some of the constraint equations developed by Pacini and Shapiro for the parameters of the proposed binary system. The June 8 upper limit of 1.7% of the peak flux leads immediately to a lower limit of  $\sim 0.8$  for the orbital eccentricity, as compared with the value of 0.4 derived by Pacini and Shapiro. This higher value of eccentricity also does not seem to be excluded by the theory developed by Wheeler *et al.*<sup>9</sup>, though we note that practically any value for the final eccentricity may be predicted, given a suitable (and not impossible) choice of initial conditions

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## New X-ray measurements of the Crab spectrum in the range 26 keV–1.2 MeV

THE Imperial College scintillation detector on Ariel V made measurements of the X-ray spectrum of the Crab Nebula in 1975 April and September. The detector consists of an  $8\text{ cm}^2 \times 4\text{ cm}$  CsI(Na) crystal actively collimated to  $8^\circ$  FWHM, and measures X rays in 15 logarithmically spaced energy channels in the range 26 keV–1.2 MeV. The detector axis is inclined  $3^\circ$  to the spacecraft spin axis, which in turn is normally offset by  $\sim 3^\circ$  from the source being viewed. Source counts are thus spin modulated, allowing the background to be subtracted. A residual modulation is further removed by changing the direction of the offset to the source and observing the resulting phase change in the modulation. This observational technique ensures all sources of background are eliminated, the modulation characteristics having been previously determined by laboratory calibration.

Counting rates were then deconvolved from the detector response by considering the effects due to window thickness, crystal dead layer and K shell X-ray escape, as well as the

various statistical processes that occur in the detection sequence from the incident photon to the phototube output.

The 1975 observations of the Crab Nebula were first analysed separately, but as no significant variation was observed they have been combined in Fig. 1. Error bars represent  $1\sigma$  counting statistics, which we believe dominate any other systematic errors. Also plotted on this figure are data below 20 keV obtained from a rocket flight by Toor and Seward<sup>1</sup> in 1970, and results above 1 MeV from balloon flights by Baker *et al.*<sup>2</sup> in 1972 and Schonfelder *et al.*<sup>3</sup> in 1974.

Though there have been many studies of the Crab Nebula by balloon and rocket experiments observing at  $< 100\text{ keV}$  (see ref. 2) and some limited observations above 1 MeV, there has previously been a noticeable lack of any accurate observations between these two ranges.

The dotted line shown in Fig. 1 is the best fit given by Toor and Seward to all available data and extrapolated from their limit of  $\sim 50\text{ keV}$  up to 10 MeV. As can be seen, the fit to the Ariel V results is excellent up to 500 keV and lies within one s.d. of the final point at 840 keV. Our results are thus consistent with a power law extrapolation to beyond 1 MeV, and hence with Schonfelder's upper limits. On the other hand, if Baker's results are correct, this would imply a rather abrupt hardening of the spectrum at about 800 keV, with a consequent softening above 10 MeV to bring the spectrum into agreement with spark chamber measurements by Kniffen *et al.*<sup>4</sup> at 50–100 MeV.

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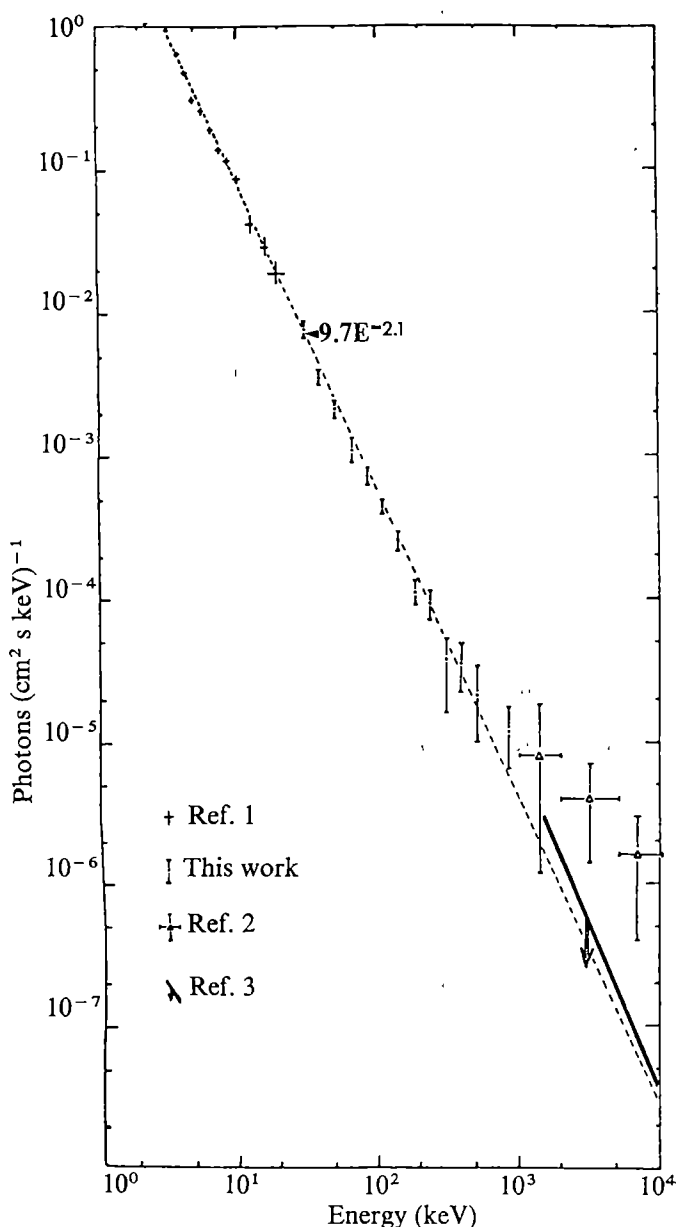
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Fig. 1 Observations of the Crab Nebula from 1 keV to 10 MeV.



## Meteor radar rates and the solar cycle

A WORLDWIDE increase in meteor echo rates in 1963 was observed in New Zealand<sup>1</sup>, Canada and Sweden and has been widely discussed in the literature<sup>2–8</sup>. From radar observations in 1953–66 I reported<sup>9</sup> a long term variation in the echo count rate with a peak occurring in 1963 near the solar minimum. Those observations also showed that the height of first appearance of meteors from a given shower had remained nearly constant at  $\sim 110\text{ km}$ , whereas the average endpoint height had risen by  $\sim 11\text{ km}$  from 1956 to 1963. It was thus evident that the 1963 peak in the meteor echo count rate was of atmospheric origin. We report here further observations, and propose that the phenomenon can be explained by a solar controlled variation of the atmospheric density gradient at the meteor ablation level, probably caused by a variation in the solar X-ray flux.

My observations were made at the Onsala Space Observatory in the period 1953–72. Visual and radar recordings of meteors have been regularly made in August since 1953. A radar control run in September was operated intermittently from 1953 to 1959 and has been regularly performed since 1962. The radar equipment records echoes of duration  $\geq 0.02\text{ s}$  corresponding to meteors brighter than about fifth zenithal magnitude. The experimental technique has been described elsewhere<sup>5,10</sup>.

Figure 1a depicts the mean night-time hourly rate of meteor radar echoes of all durations, as observed from 1953 to 1972 (1954 is missing and 1971 not yet reduced). A long term variation in the August meteor rate curve is evident. The operational run in August includes the period of maximum activity of the Perseid meteor shower, but the length of the run is such that



the observed total echo rates may be considered a measure of sporadic meteor activity. It is also evident that total echo rates for the control period in September, when no prominent shower is active, exhibit similar temporal variations. It is therefore concluded that the observed time variation in meteor rates is representative of meteor rates in general. Figure 1 suggests a variation by a factor of  $\sim 2$  in the echo rate curve with peaks primarily confined to times near solar minimum, and further compares the total radar rate curve with the mean endpoint height and trail length of meteors of a given shower. These meteors represent a homogeneous sample with respect to velocity, angle of incidence and composition. It is evident that the meteor endpoint heights and meteor radar rates exhibit similar temporal variations. There is further an inverse relationship between radar rates and the mean trail length of meteors (Fig. 1c). We found that the echo amplitudes showed a temporal variation similar to that of the radar rates.

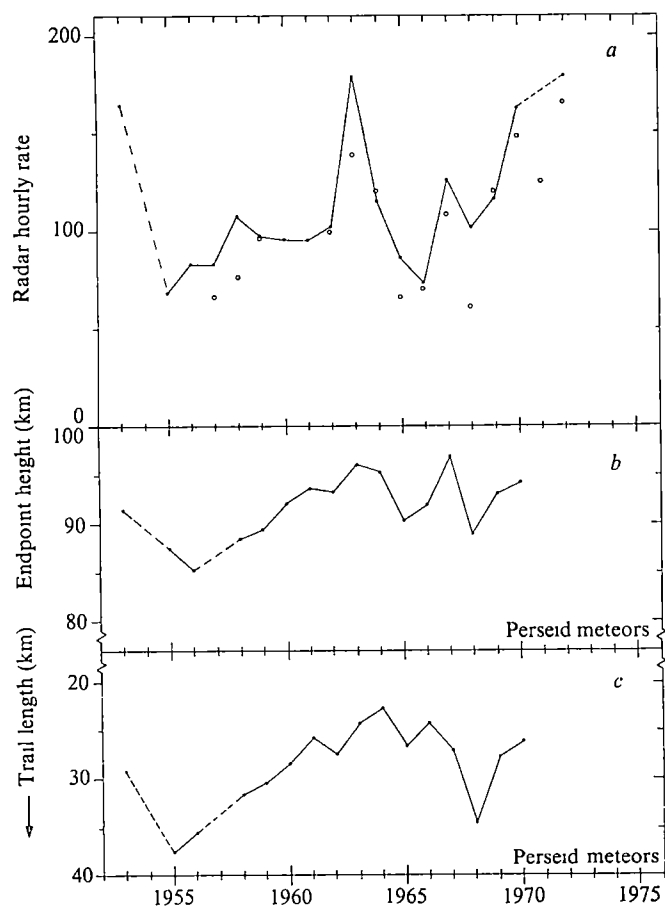


Fig. 1 Comparison between meteor echo rates, meteor endpoint heights and meteor trail lengths. *a*, Mean night-time hourly rate of meteor radar echoes of duration  $> 0.02$  s observed in August (●—●) and September (○) during the period 1953–72. Radar observations were made at a frequency of 33 MHz. *b*, Mean endpoint height of Perseid meteor trails determined from combined radar and visual observations. *c*, Mean trail length of Perseid meteors.

Figure 1 suggests a dependence of radar rates on position in the solar cycle. The phase of the solar cycle variation is analysed in the periodogram in Fig. 2. The zero epoch in the diagram is that of the sunspot maximum. It is evident that the highest radar rates are observed 4–6 yr after a solar maximum and the lowest, 1–2 yr before a solar maximum. This result is consistent with those of an analysis of visual meteor rates (1844–1933) published by Bumba<sup>11</sup>. The scale in Fig. 2 may be approximately referred to solar minimum by subtracting 6.5 yr. One may therefore conclude from Fig. 2 that the phase of the radar rate curve is such that peak meteor rates are observed 1–2 yr before a solar minimum.

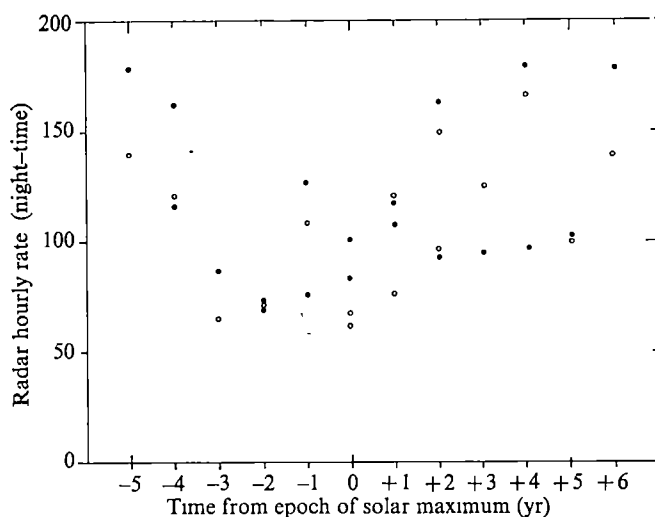
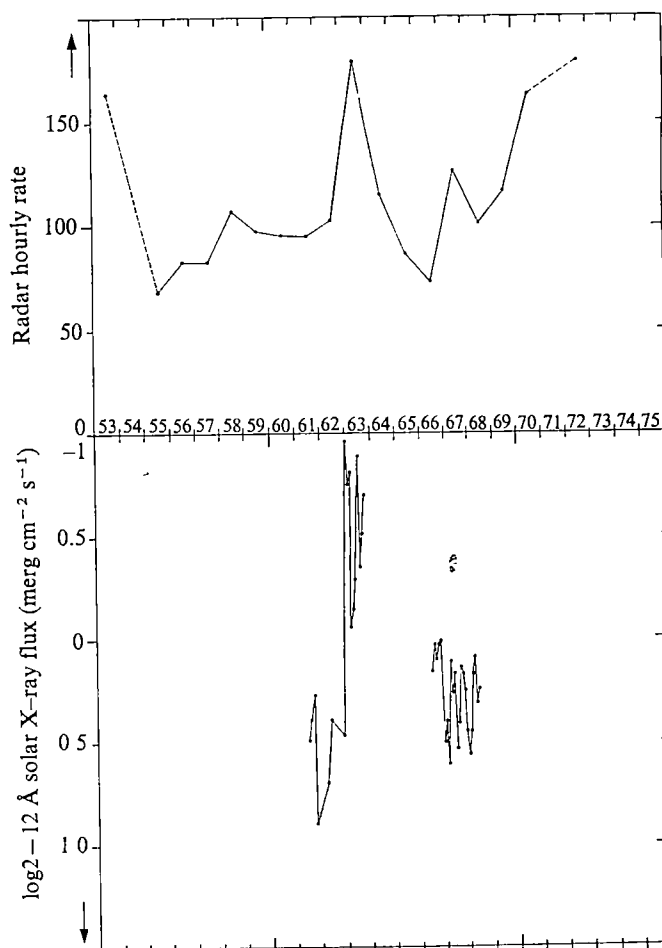


Fig. 2 Variation of meteor radar rates with position in the solar cycle. Epochs of sunspot maxima are 1957.9 and 1968.9. All time differences in the diagram have been rounded off to an integer number of years.

A long term variation in radar rates, meteor trail length and radar echo strength may be qualitatively explained if it is assumed that the density gradient of the neutral atmosphere between 85 and 110 km varies periodically with position in the solar cycle. If the density gradient is increased near solar minimum, the meteor ablation process will occur over a shorter

Fig. 3 Mean night-time hourly rate of meteor radar echoes (upper diagram); mean monthly non-flare 2–12 Å solar X-ray flux after Wende (lower diagram).



length of trail and a larger electron line density will result from a particular meteoroid. This will increase the echo signal strength and thus allow smaller meteors to come within the detection threshold of the radar, thereby producing an increase in the total echo rates.

The observed temporal variation of meteor trail length provides information on the variation of the atmospheric density scale height. The length of a meteor trail may be written<sup>12</sup>

$$L = cH \sec z_R \quad (1)$$

where  $L$  and  $H$  are meteor trail length and atmospheric scale height, respectively;  $z_R$  is the zenith distance of the radiant and  $c$  is a numerical constant, which depends on mass, velocity and limiting visual magnitude. Insertion of  $H = 5.7$  km,  $L = 28.2$  km and  $\sec z_R = 1.40$  gives  $c = 3.53$ . Observed maximum and minimum values of trail length were 37.5 km for 1955 and 22.7 km for 1964. On the basis of the data it is tentatively suggested that the average density scale height at the meteor ablation level has varied from about 7.5 km in 1955 to 4.5 km in 1964.

The heating mechanism required to produce a solar cycle effect in the atmospheric density gradient at the meteor ablation level is not properly understood. A long term variation in the X-ray and particle flux emitted from the Sun seems to be the most probable cause. Kreplin<sup>13</sup> has summarised the available rocket and satellite data on the solar X-ray flux and found that for wavelengths of 8–20 Å, which penetrate to, and are absorbed in, the meteor zone, the solar cycle variation may exceed a factor of 100. A very variable heat source thus exists in the meteor ablation zone.

Wende<sup>14</sup> has investigated the solar cycle variation in the integral, non-flare, solar X-ray flux between 2 and 12 Å, as measured by the spacecrafts Injun 1, Injun 3, Explorer 33 and Explorer 35. Figure 3 compares the monthly means of X-ray flux with the radar rate curve. Although the X-ray data cover only three periods in 1961–68, the data suggest an inverse correlation between meteor rates and solar X-ray flux. Of particular interest is the rather abrupt decrease in the solar X-ray flux observed at the beginning of 1963 simultaneously with the reported worldwide increase in meteor radar rates. The 1963 increase in meteor radar rates might thus reasonably be ascribed to a change in the atmospheric density gradient due to a sudden drop in the solar X-ray flux.

Existing theories of meteor ionisation generally assume an isothermal atmosphere, and do not take into account spatial and temporal variations in temperature in the meteor ablation zone. Therefore no detailed theoretical explanation of the observed long term variations in meteor radar rates and heights is available. Observed changes in radar echo amplitudes of bright, visual meteors<sup>9</sup> are far larger than what one would expect from meteor ionisation theory.

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## Self reversal of thermoremanent magnetisation in basalts and global lunar magnetism

RYALL and Ade-Hall have shown experimentally<sup>1</sup> that prolonged heating of pillow basalts in suitable conditions results in the exsolution of a 'daughter' phase from the host titanomagnetite. They suggest that in an applied field the daughter phase acquires a chemical remanent magnetisation (CRM) when it grows to the single domain size and that on cooling, the 'mother' or host phase is subjected to an interaction field from the daughter phase, which, they suggest, could outweigh the effect of the external field and result in a net reversed moment of the sample. They suggest that magneto-static or possibly exchange interaction might be responsible.

By using magnetostatic theory which has already been applied to a simple model of a two-phase titanomagnetite particle—and, on a rather larger scale to the problem of the residual moment of the Moon—it can be shown that a magneto-static interaction can indeed explain their results.

Consider a simple model of a two-phase particle as shown in Fig. 1. The daughter phase is assumed to be spherical, of radius  $a$ , positioned at the centre of a spherical host phase of radius  $b$ . If the relative permeabilities are  $\mu_{1r}$  and  $\mu_{2r}$  respectively, and if the daughter phase carries a CRM of  $M_1$  per unit volume, where  $M_1 = M^*_1 - H_d$  ( $\mu_{1r} - 1$ ), ( $H_d$  is the demagnetising field within phase 1, and  $M^*_1$  is the CRM if  $H_d = 0$ ), then the external moment  $m_e$  is given by<sup>2</sup>

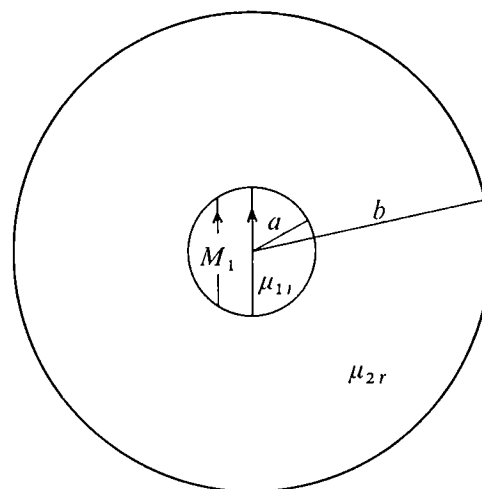
$$m_e D^2 / 4\pi b^3 = 3\sigma \mu_{2r} M^*_1 (\mu_{1r} + 2\mu_{2r})(\mu_{2r} + 2) \quad (1)$$

provided that  $\sigma \ll 1$  where  $\sigma = (a/b)^3$ , and that the permeability of the medium in which the moment is measured ( $\mu_{3r}$ ) is unity. For small values of  $\sigma$ ,  $D = (\mu_{1r} + 2\mu_{2r})(\mu_{2r} + 2)$ . On cooling, the host phase (Fig. 2) will acquire a thermoremanent magnetisation (TRM) in the field produced by the internal dipole (attributable to the CRM) and if  $k$  represents the efficiency of the TRM acquisition process (that is,  $M_{\text{TRM}} = kH$ ) then the external moment, attributable to the spherical shell alone<sup>3</sup> is  $m_i$  where

$$m_i D^2 / 4\pi b^3 = 6k\sigma M_1^* (\mu_{1r} - \mu_{2r}) \quad (2)$$

If  $\mu_{2r} = \sqrt{\mu_{1r}}$ , the external moment attributable to the host phase is thus zero. If the volume of the host particle is multiplied by  $\sim 10^{30}$  the body becomes of planetary dimensions,

Fig. 1 Model of two-phase particle (radii  $a$  and  $b$ ). The inner 'daughter' phase (radius  $a$ ) carries a CRM/ $M_1$  (see text). The moment,  $m_e$ , of the particle is given by equation (1).



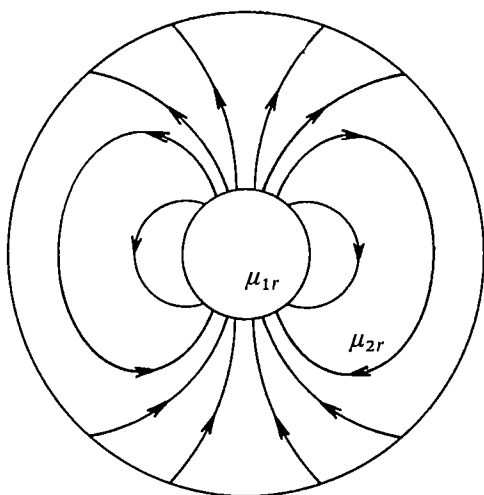


Fig. 2 The component of TRM (schematic) acquired by a host phase in field of a daughter phase. The moment,  $m_i$  attributable to this component is given by equation (2). See text for notation.

and if  $\mu_{1r} = \mu_{2r} = 1$ , then  $m_i = 0$ . This result corresponds to that derived for the case of the Moon by Runcorn<sup>4</sup>, who showed that if the outer shell of the Moon were magnetised in the field of an internal dipole which has now disappeared, the present lunar dipole moment should be zero. Since  $\mu_{2r}$ , however, must always exceed unity if a TRM is acquired and probably exceeds  $\mu_{1r}$  in the Moon, a small negative lunar dipole moment should be observed<sup>3</sup>.

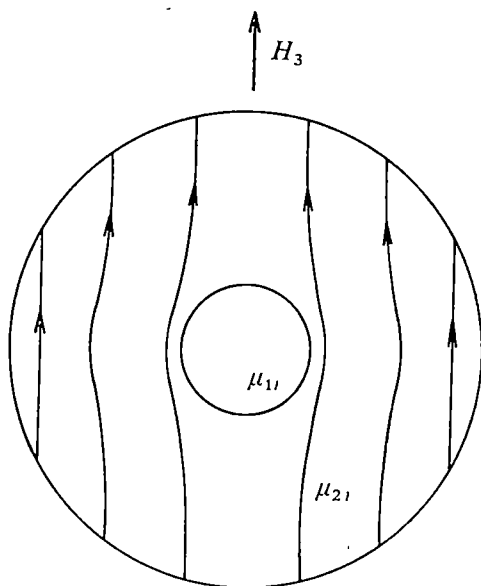
Similar theory applied to the component of TRM which the host phase acquires as it cools in the external field  $H_3$  (Fig. 3), enables the following equation to be derived for the external moment  $m_e$ .

$$m_e D^2 / 4\pi b^3 = 3kH_3(\mu_{1r} + 2\mu_{2r})^2 \quad (3)$$

The total moment  $m$  of the two-phase particle is thus the sum of three terms given by equations (1)–(3).

The only negative contribution to the moment comes from the second term, and then it arises only if  $\mu_{2r}^2 > \mu_{1r}$ . This condition is almost certainly fulfilled if, as Ryall and Ade-Hall suggest<sup>1</sup>, the CRM resides in a single domain, daughter phase for which  $\mu_{1r}$  that is,  $(dB/\mu_0 dH) = 1$ .

Fig. 3 The component of TRM (schematic) acquired by a host phase in an external field  $H_3$ . The moment,  $m_e$ , attributable to this component is given by equation (3). See text for notation.



A second condition which must hold if  $m$  is to be negative is that  $|m_i| > m_e$ , and if  $\mu_{2r}$  is assumed to be  $\gg 1$ , then  $\sigma M^*_1 > 2H_3$ . A typical titanomagnetite has a spontaneous magnetisation of the order of  $10^5 \text{ A m}^{-1}$  (100 gauss), so putting  $M^*_1 (= M_1 \text{ for a single domain grain with } \mu_{1r} = 1)$  equal to  $10^5 \text{ A m}^{-1}$  and since  $H_3 \approx 80 \text{ A m}^{-1}$  (1 oersted), then  $\sigma > 1.6 \times 10^{-3}$ , which corresponds to a ratio  $a/b$  of  $\sim 0.1$ . Thus, only about 0.16% by volume of daughter phase is required to produce reversal.

The third condition which is necessary is that  $|m_i| > m_e$ ; thus for  $\mu_{2r} \gg 1$ ,  $k > \mu_{2r}$ . To see whether this condition is reasonable it is necessary to consider how the TRM process operates. Before the host phase cools (assumed multidomain) but while it is still below the Curie point, the induced magnetisation,  $M$ , at any point is  $(\mu_{2r} - 1)H$ , where  $H$  is the local field, and where, if  $\mu_{2r}$  is high,  $M \approx \mu_{2r}H$ . On cooling, this magnetisation becomes blocked in as domain walls become immobilised in local potential wells. On further cooling, the spontaneous magnetisation will change from the value  $M_s(T_B)$  at the blocking temperature  $T_B$ , to  $M_s(T_0)$  at room temperature,  $T_0$ . Thus,  $k = \mu_{2r} M_s(T_0) / M_s(T_B)$ . Since spontaneous magnetisation usually increases on cooling, then  $k > \mu_{2r}$ , and that condition can be satisfied. There are, of necessity, many simplifications in this argument. The factor  $\mu_{2r}$  is assumed to be constant though it will in practice vary with temperature. Each host particle will contain many inclusions of daughter phase which are represented here by a single inclusion which has the same properties as the individual inclusions and a volume equal to the sum of the individual volumes. Spherical symmetry is also assumed. The assumption that  $M_{\text{TRM}} = kH$  will break down near the surface of the single domain inclusion since the local field there will be well above the low field range where a linear dependence is normally found. Nevertheless, provided  $\sigma$  is small, the regions where the nonlinear effects operate will also be of relatively small volume and may possibly be neglected.

In spite of all the simplifications, reverse TRM of the type found by Ryall and Ade-Hall can be explained on magnetostatic theory using the model described here. Before the daughter phase forms, the moment is given by equation (3). As the daughter phase increases in size through the single domain range,  $\sigma$  increases and the second term (equation (2)), which dominates the first (equation (1)), causes a decrease in moment until reversal occurs. Once the daughter phase exceeds the single domain size,  $M^*_1$  decreases and  $\mu_{1r}$  will increase so that the negative contribution will diminish and the moment will revert to normal, in line with experimental observations<sup>1</sup>.

It is interesting that the second term (equation (2)) which governs whether or not reversal occurs is the contribution from that component of remanence in the shell which was produced by the field of the central magnetised inclusion. This would also be the term describing the present lunar global dipole moment were the shell originally magnetised in the field of an ancient dipole which has now disappeared<sup>3</sup>.

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## Origin of corundum-normative intrusive and extrusive magmas

MANY intrusive and extrusive calc-alkaline suites show a continuous trend from diopside-normative basic magma to corundum normative acid magmas, that is, with an increasing  $\text{SiO}_2$  content there is an exceptional decrease in the Ca:Al ratio

of the magma. A number of explanations have been proposed for these trends, including secondary alteration, vapour phase transfer, assimilation, crustal remelting, hydrous melting of the upper mantle and the fractional crystallisation of a variety of phases. We demonstrate here that corundum-normative magmas can be produced by the fractional crystallisation of a hornblende amphibole from calc-alkaline magma under moderate water pressure.

A large proportion of granitoid rocks are corundum-normative<sup>1</sup> and Chayes<sup>2</sup> found that 18% of 1,775 andesite analyses he compiled were also corundum-normative. By contrast, rocks of tholeiitic affinity are virtually always diopside-normative, even for the acid differentiates. This difference in normative mineralogy between calc-alkaline and tholeiitic suites has not been adequately explained.

Several calc-alkaline trends, which range from basaltic, through andesitic to dacitic and rhyolitic (gabbroic to ademetilic for intrusive suites) are plotted on Fig. 1, showing the transition from diopside-normative to corundum-normative. All the suites show a systematic trend of decreasing diopside content, eventually becoming corundum-normative with increasing  $\text{SiO}_2$ . The exact value of the  $\text{SiO}_2$  content at which the rocks become corundum-normative varies considerably from about 56% for the Borrowdale volcanics<sup>3</sup> to 70% for the Sierra Nevada batholith<sup>4</sup> and the Cascades volcanics<sup>5</sup>. All the trends, however, appear to be subparallel.

Various models have been proposed for the production of corundum-normative magmas. They include:

**Secondary alteration.** The leaching of alkalis from pumice fragments after eruption could produce corundum-normative compositions<sup>6</sup>. It is, however, unlikely that 18.4% of all andesites<sup>2</sup> could be so affected. It is also remarkable that no tholeiitic suites contain leached compositions. The trends on Fig. 1 are regular. Leaching of alkalis would not be expected to produce as regular an alteration. Alkali leaching from intrusive rocks is very unlikely, and yet they show the same trend as volcanics. We do not accept alkali leaching as a general model.

**Vapour phase transfer.** Interaction of an aqueous vapour phase with magma could remove alkalis<sup>7</sup>. This could, however, only happen once the magma has become water saturated. The evidence does not support the idea that most calc-alkaline suites are water saturated. We do not accept it as the general mechanism for producing corundum-normative magmas.

**Assimilation.** Contamination of magma could produce a corundum-normative product, as has been demonstrated for the Taupo Volcanics, New Zealand<sup>8</sup>. But  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios do not indicate any significant contamination of two of the suites plotted in Fig. 1. The Cascades Volcanics<sup>9</sup> and the Lesser Antilles Suite<sup>10</sup> are not contaminated by crustal material. So we do not favour contamination as a satisfactory way of explaining all corundum-normative magmas.

**Crustal remelting.** Presnall and Bateman<sup>4</sup> appealed to a process of fusion of andesitic crustal material to explain the chemical variation of the Sierra Nevada batholith (although they considered only the granitic component of the rocks). We note that partial melting of andesitic crust leaving an amphibole-rich residue could produce corundum-normative liquids, but corundum-normative magmas are observed where there is no significant continental crust, for instance, in the Lesser Antilles<sup>11</sup>. Thus remelting of continental crust cannot be a generally applicable model.

**Melting of hydrous peridotite.** Kushiro and Yoder<sup>16</sup> have suggested that corundum-normative magmas could be produced by the melting of hydrous peridotite. That model does not, however, explain the continuous trend of compositions from basic to acidic. Furthermore, the model requires unrealistically high geothermal gradients for the mantle. The data were obtained under 10 kbar pressure<sup>12</sup>, and Kushiro<sup>13</sup> subsequently showed that partial melts from a hydrous peridotite were not corundum-normative at 20 kbar. Therefore, corundum-normative magmas would have to be formed at less than 20 kbar (60 km). Probable temperatures at this depth under

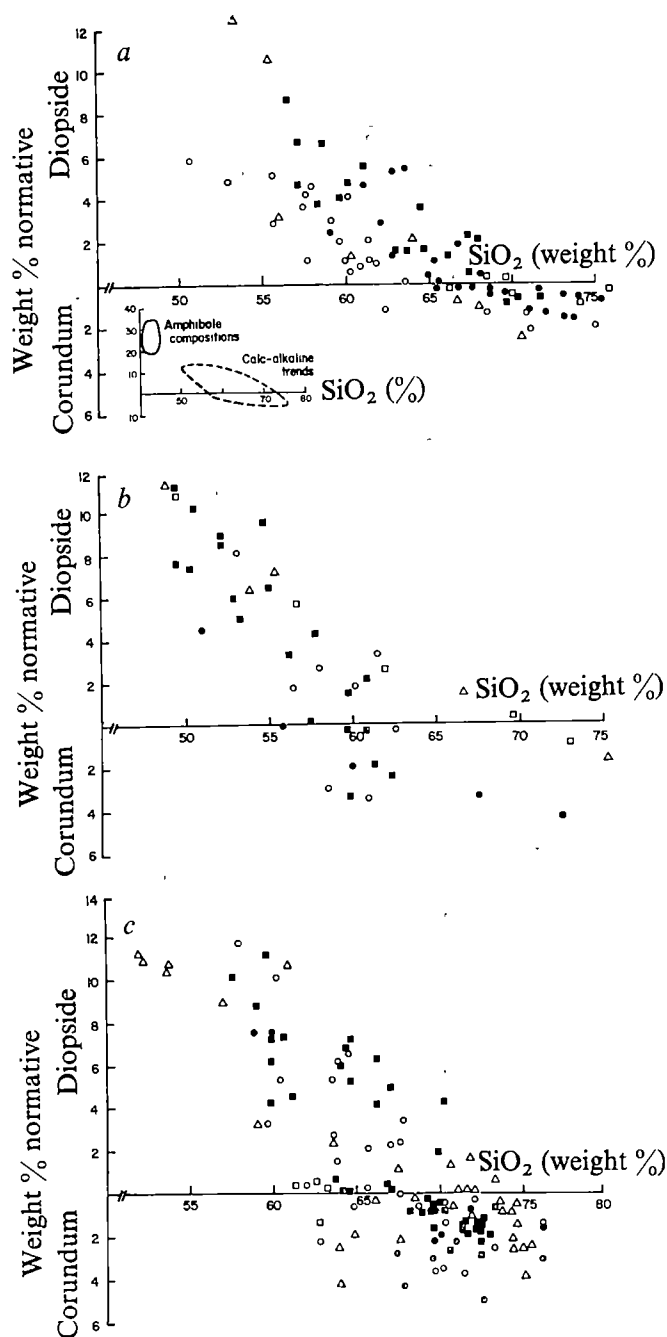


Fig. 1. Plots of normative diopside and normative corundum against  $\text{SiO}_2$ . a, Calc-alkaline intrusive magmas: ●, Sierra Nevada<sup>4</sup>; ■, Ben Nevis<sup>19</sup>; ○, Alaska<sup>20</sup> (Jurassic only); □, Namibia<sup>21</sup>; △, Garabal Hills<sup>22</sup>. b, Extrusive calc-alkaline magmas from various tectonic environments: ●, Borrowdale<sup>3</sup>; ■, Lorne<sup>23</sup> (basalt to andesite only); ○, St Kitts<sup>11</sup>; □, Cascades<sup>5</sup>; △, Japan<sup>14</sup>. c, Lower Palaeozoic Appalachian rocks from Newfoundland<sup>24</sup>: ●, Middle Ridge; ■, Holyrood; ○, Middle Brook; □, Deadman's Bay; △, Mount Peyton (Inset on a: range of amphibole compositions synthesised from basic to andesitic compositions (see ref. 17) compared with the trends of the intrusive and extrusive calc-alkaline magmas.)

both continental and oceanic crust are too low to partially melt hydrous peridotite.

**Fractional crystallisation.** Kushiro and Yoder<sup>12</sup> also proposed that the fractionation of clinopyroxene could produce a trend from diopside-normative to corundum-normative. The clinopyroxene they synthesised would, however, not produce the trends observed in Fig. 1.

Kuno<sup>14</sup> has suggested that the fractionation of magnetite-rich gabbro could produce the calc-alkaline trend. But there is a thermal divide at low pressure in the plane anorthite-forsterite-quartz<sup>15</sup> such that crystallisation to produce a corundum-normative residue from diopside-normative liquids is not

possible. This is supported by Fitton's finding<sup>3</sup> that clinopyroxene is never observed in corundum-normative compositions. So we do not believe that low-pressure crystallisation can produce corundum-normative liquids.

Green and Ringwood<sup>16</sup> have suggested that garnet pyroxenite fractionation could produce the calc-alkaline trend, but they showed that the liquidus temperatures increased for compositions more siliceous than 61% SiO<sub>2</sub>. In Fig. 1 the trends continue from 50% to over 70% SiO<sub>2</sub>.

A fourth possible fractionation model for the genesis of the calc-alkaline suite has been suggested by Cawthorn and O'Hara<sup>17</sup>. They have suggested that amphibole fractionation may produce the observed chemical variation. We have plotted in the inset of Fig. 1 various amphibole compositions which have been synthesised from basic and intermediate compositions under 5–15 kbar pressure (see ref. 17). These plot on the back projection of the calc-alkaline trends and we suggest the crystallisation of such amphiboles explains these trends satisfactorily. Slight variations in slope of the trends in Fig. 1, may be attributable to different amphibole compositions crystallising at different pressures, or possibly to the coprecipitation of amphibole and plagioclase, which may become important in the most silicic compositions<sup>17</sup>.

Pinwinski and Wyllie<sup>18</sup> have melted a series of corundum-normative granitoid rocks and shown that hornblende is the liquidus phase for most compositions. Thus, amphibole will crystallise from corundum-normative magmas and is capable of producing the characteristic trend of calc-alkaline magmas.

We therefore suggest that amphibole fractionation is the most plausible single model to explain all corundum-normative magmas (although the other models have a limited applicability).

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## Argon isotopic evolution of upper mantle

BROWN *et al.*<sup>1</sup> have treated the problem of the history of the argon degassing of the Earth, and claim to demonstrate that the <sup>40</sup>Ar/<sup>36</sup>Ar ratio in the mantle has increased from ~133, 770 Myr ago, to a value that "is at present not greater than the atmospheric ratio (295.5) and is probably a little lower". Their Table 1 and Fig. 1 show the results of K–Ar isochron analyses of several suites of "mantle-derived igneous" samples. We have been unable to reproduce in detail their results from the references they cite, and conclude that the apparent trend in their Fig. 1 is an artefact of data selection and that the source of the

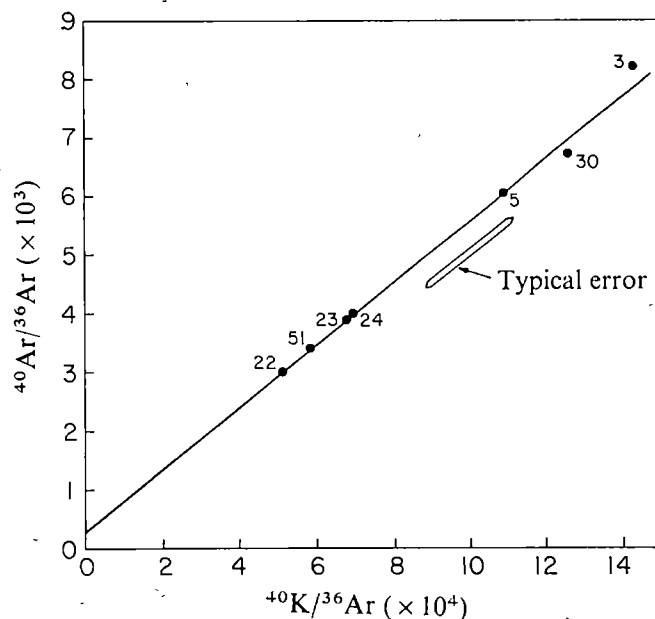


Fig. 1 A K–Ar isochron plot of group IIIA dyke data<sup>2</sup>. The slope and intercept shown are from a regression line fitted to points 5, 22, 23, 24 and 51 (see text), which gives slope =  $0.05274 \pm 0.00052$ ; an age of  $736.7 \pm 6.0$  Myr; intercept ratio =  $298 \pm 34$ .

observed argon has been misidentified. We think the <sup>40</sup>Ar/<sup>36</sup>Ar ratios in the solid earth and the atmosphere have probably increased with time. Theoretical considerations and the available literature both indicate, however, that the <sup>40</sup>Ar/<sup>36</sup>Ar ratio in the solid earth is much higher than the <sup>40</sup>Ar/<sup>36</sup>Ar ratio in the atmosphere.

The apparent trend in Fig. 1 of Brown *et al.*<sup>1</sup> is mainly a function of the oldest datum, number 18, based on seven K–Ar analyses of the "group IIIA dike" samples<sup>2</sup> from the Beartooth mountains. These data are shown in Fig. 1 on a plot of <sup>40</sup>Ar/<sup>36</sup>Ar against <sup>40</sup>K/<sup>36</sup>Ar. A regression<sup>3</sup> of all seven data points using a correlation coefficient of 0.9 yields an age of  $745 \pm 21$  Myr and an ordinate intercept or

Fig. 2 A plot of (<sup>40</sup>Ar/<sup>36</sup>Ar)<sub>i</sub> against age for the suite of samples chosen by Brown *et al.* The dashed line shows the present atmospheric <sup>40</sup>Ar/<sup>36</sup>Ar ratio of 295.5. The error bars shown are one standard deviation (1σ) when the error is larger than the points. The small solid data points are from the calculations of this work. The open data points are those data from Brown *et al.* which differ significantly from ours. Points 15 and 16 of Brown *et al.* have been omitted because the data on which the points are based are not available in the scientific literature and points 2–7 have been omitted for reasons outlined in the text. The large solid data points shown for points 17 and 18 are the values shown in italics in Table 1 and are our best estimates of the trapped gas in these two systems.

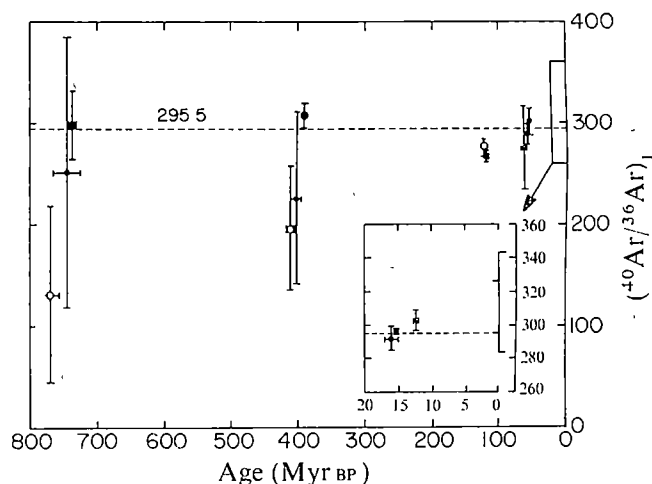




Table 1 'Measured' variation in  $(^{40}\text{Ar}/^{36}\text{Ar})_i$  ratios

Point	$(^{40}\text{Ar}/^{36}\text{Ar})_i$		Age (Myr)		Ref.	Comments
	(This work)	(Brown <i>et al.</i> )	(This work)	(Brown <i>et al.</i> )		
1	283.8→342.4	282.9→294.8	Historic	Historic	10	Historic Basalts
2	295.1→326.0	292.1→320.4	Historic	Historic	9	'SP' single basalt flow
8	302.6±6.2	302±6	12.35±0.16	12.4±0.3	20	East Iceland Volcanics
9	296.4±0.6	295±3	15.20±0.05	15.3±0.2	21	Steens Mountain basalts
10	291.8±7.6	293±19	16.0±1.0	15.8±1.3	20	North-west Iceland Volcanics
11	302±11	289±21	51.7±21	53.1±2.5	22	Top of middle series Faroe Island basalts
12	274±42	263±21	61.7±5.6	64.8±3.1	22	Base of middle series Faroe Island basalts
13	287.9±9.9	287±9	55.2±1.2	55.4±1.2	22	Base of lower series Faroe Island basalts
14	266.4±6.2	275±8	117.0±3.8	119±4	5,6	Quartz diorite minerals
15	*	183±70	*	410±4	?	Etive Granites
16	*	224±37	*	415±4	?	Lorne Lavas
17	226±85 308±12	196±61	401.1±6.3 389.5±1.0	411±3	4 and pers comm.	Argyll biotites
18	252±133 298±34	131±88	745±21 736.7±6.0	770±14	2	Dolerite dikes

\*Data contained in a thesis not available to us.

Our results were obtained using a 1969 York<sup>3</sup> regression program. The correlation between errors in the ordinate and abscissa of the isochron plots was assumed to be zero except as noted in the text. All of the listed errors are 1 s.d. (1σ). The following values were used for the decay constants and abundance of  $^{40}\text{K}$ :  $\lambda_{\text{EC}} = 0.585 \times 10^{-10} \text{ yr}^{-1}$ ,  $\lambda_{\beta} = 4.72 \times 10^{-10} \text{ yr}^{-1}$ ,  $^{40}\text{K}/\text{K} = 1.19 \times 10^{-4} \text{ mol mol}^{-1}$ .

initial  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio of  $252 \pm 133$ . Five of the samples (numbers 5, 22, 23, 24, and 51), however, form a good linear array, and most of the error comes from the two most radiogenic samples (numbers 3 and 30). A regression of the five colinear data yields an age of  $737.7 \pm 6.0$  Myr, and an intercept of  $298 \pm 34$ . We feel that this represents the best estimate of the age and intercept of this suite. Sample 3 contains slightly more radiogenic Sr than the other samples, which may indicate a small amount of crustal contamination. A regression of six samples (omitting number 3) yields an age of  $715 \pm 12$  Myr and an intercept of  $411 \pm 72$ . Brown *et al.*, however, omitted sample 30 and retained sample 3 in their regression, and we have found no objective reason for doing this.

Data from Argyll biotites (ref. 4 and a 1973 Oxford thesis (ref. 30 in Brown *et al.*)) are the source of data points 15, 16 and 17. Because the referenced thesis is unavailable we are unable to check points 15 and 16. We have, however, obtained the unpublished data (J. F. Brown, personal communication; R. J. Pankhurst, personal communication) included with the Argyll data for point 17. The regression of these data yields an age of  $401.1 \pm 6.3$  Myr and an intercept of  $226 \pm 85$ . Since the  $^{39}\text{Ar}$  content in triplicate argon analyses of one of the samples (number 4 in ref. 4) shows almost as much variation, a factor of 1.6, as do all of the data combined, a factor of 1.9, it is clear, however, that these data define mixing lines, not isochrons. The triplicate analyses of sample 4 yield the best defined mixing line with an intercept of  $308 \pm 12$  and an 'age' of  $389.5 \pm 1.0$  Myr. This mixing line is the best estimate of the trapped argon in this system. The system is clearly disturbed, however, since a regression of the data obtained by averaging the replicate analyses of each sample yields an age of  $424.1$  Myr and an impossible intercept of  $-98 \pm 169$ .

Data point 14 is taken from Hayatsu and Carmichael's<sup>5</sup> treatment of data from mineral separates from a pyroxene quartz diorite in the Sierra Nevada of California<sup>6</sup>. The minerals show evidence of alteration<sup>6</sup> and the hornblende datum lies above the line defined by the other minerals (see Fig. 5 of ref. 5). This set of minerals clearly represents a disturbed system. Point 14 also demonstrates graphically the danger of using someone else's K-Ar data for isochron plots. Brown *et al.* apparently assumed, as did Hayatsu and Carmichael<sup>5</sup>, that the column labelled " $^{40}\text{Ar}/^{36}\text{Ar}_{\text{total}}$ " in Table 2 of ref. 6 means radiogenic  $^{40}\text{Ar}$  divided by atmospheric  $^{40}\text{Ar}$  plus radiogenic  $^{40}\text{Ar}$ . Kistler (personal com-

munication) indicates that " $^{40}\text{Ar}_{\text{total}}$ " contains a significant component of  $^{40}\text{Ar}$  from the isotope dilution spike. After correcting for this component we obtain an age of  $117.0 \pm 3.8$  Myr and an intercept of  $266.4 \pm 6.2$  for the quartz diorite minerals—but the hornblende datum still plots off the line.

The rest of the data, points 1–13, have intercepts indistinguishable from the present atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  value. Some require comment, however. We calculate higher intercepts and lower ages for points 11 and 12 than do Brown *et al.* The amounts of  $^{39}\text{Ar}$  in replicate analyses involved in points 8 and 10 show wide variations which indicate mixing lines.

Points 3–7 are from analyses of the Auckland volcanic field by McDougall *et al.*<sup>8</sup>. They do draw isochron diagrams but go on to conclude, "these linear arrays of data must be interpreted as indicating that the amount of excess Ar is nearly constant for a given volcano, and that the apparent isochrons are a result of this fact, together with the small variation of K in lavas from a single volcano" (p. 1517).

The data are from mixing lines, not isochrons, and do not belong in a compilation of 'initial'  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios. The clearest case is the data from the Rangitoto volcano. As McDougall *et al.*<sup>8</sup> state, for Rangitoto "the radiocarbon, geological and botanical evidence unequivocally shows that it was active and was probably built in the last 1,000 years" (p. 1485). K-Ar data from Rangitoto define two apparent isochrons and two separate fits are made to one of these<sup>8</sup>, but the 'ages' which accompany these lines are ~500 and 200 times too large.

Point 2 is from work on the SP flow in the San Francisco volcanic field in Arizona<sup>9</sup>. The distribution of  $^{36}\text{Ar}$  through the flow strongly suggests that the  $^{36}\text{Ar}$  came from the atmosphere and not the mantle.

Point 1 is from work on modern surface volcanics<sup>10</sup>. Three samples (out of 27) had  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios less than the atmospheric value, but Krümmenacher<sup>10</sup> demonstrated that these low values were due to mass fractionation effects on argon of atmospheric composition trapped in the lava and "not due to a mixture with primordial argon, that is depleted in  $^{40}\text{Ar}$ ". Brown *et al.* report only three low  $^{40}\text{Ar}/^{36}\text{Ar}$  data as their point 1. Baksi<sup>11</sup> has confirmed the basic observation that mass fractionation is often the dominant process in young volcanic rocks.

Our calculations indicate that Fig. 1 of Brown *et al.* should be replaced by Fig. 2 of this paper. The line shown

in their Fig. 1 is a physical impossibility since it extrapolates to  $-666$  at  $4,500$  Myr. We interpret our Fig. 2 as evidence that most, if not all, of the trapped argon in the samples shown in Table 1 and Fig. 2 is modern atmospheric argon which has been added to the samples during recent weathering and/or laboratory manipulation. There is an additional caveat. Shafiqullah and Damon<sup>12</sup> have recently discussed K/Ar isochrons and conclude that in geologically realistic situations K–Ar isochrons tend to be considerably more complicated than Rb–Sr diagrams, and should be used with great caution.

The best available samples of mantle gases are those trapped in the quenched rims of submarine basalts. These quenched rims of recent submarine pillow lavas contain argon with  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios higher than the present-day atmospheric ratio<sup>13,14</sup>. It has been reported that the elemental ratios of He, Ne, Ar, Kr and Xe in some quenched rims are not atmospheric<sup>15,16</sup>. This is interpreted as evidence that the quenched rims contain primordial rare gases from the mantle. In samples identified as containing mantle gases the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio varies from 358 to 88,000. Ozima and Kudo<sup>17</sup> have argued that the present mantle  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio is approximately 2,000. We believe that most workers would classify submarine basalts as 'mantle-derived igneous' rocks.

In summary, we can see no evidence for mantle argon with a  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio  $<295.5$ . Data not considered by Brown *et al.* strongly indicate that the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio in the mantle is  $\geq 295.5$ .

Brown *et al.* assume that "not more than 5% of crustal argon has been released to the atmosphere, and perhaps as little as 1%". They then calculate the amount of  $^{40}\text{Ar}$  accumulated from K in the crust (39% of the Earth's total inventory of K in their model) in  $4,500$  Myr, and release 5% of that  $^{40}\text{Ar}$  in the atmosphere. Brown *et al.* implicitly assume that the entire crust has been in existence for  $4,500$  Myr and has a current average K–Ar age of  $4,412$ – $4,483$  Myr. This assumption violates all of the current theories of continental growth and is completely incompatible with what is known about continental age provinces<sup>19</sup>. While some argon derived from melting and recrystallisation might be trapped in the crust, the young average age of the continental crust,  $\sim 1,100$  Myr, implies that most of the argon is degassed during uplift, weathering, metamorphism (radiogenic  $^{40}\text{Ar}$  is found in natural gases<sup>20</sup>), continental growth, and recycling in subduction zones. The fact that 5% of the crust is now sediments certainly does not mean that only 5% of crustal argon has been released to the atmosphere.

All of the detailed calculations of argon evolution of which we are aware yield mantle  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios higher than the corresponding atmospheric ratio throughout time. No matter what the initial partitioning of Ar was between the solid Earth and atmosphere, the atmosphere has no potassium  $^{40}\text{Ar}$  is produced only in the solid Earth. The  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio in the atmosphere can increase only by the degassing of the solid Earth. Degassing, however, removes both  $^{40}\text{Ar}$  and  $^{36}\text{Ar}$  from the solid Earth. While  $^{40}\text{Ar}$  continues to be produced from the decay of  $^{40}\text{K}$ , there is no mechanism which produces  $^{36}\text{Ar}$  in the solid Earth. Therefore, the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio in the solid Earth increases faster after a degassing event than it would have if the event had not occurred. Fractionating the potassium into a crust does not change the picture unless one is willing to postulate a differentiation process which removes the potassium while leaving the argon in the mantle.

In conclusion, we agree with the suggestion of Brown *et al.* that it may be possible to measure the time evolution of the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio in the mantle using K–Ar isochron techniques. If such an evolutionary history can be obtained, it will provide an important boundary condition on petrogenetic and thermal models of the Earth. A similar evolu-

tionary history for the atmosphere may also be obtainable and would be equally important. We have shown, however, that Brown *et al.* have not measured the evolution of the mantle  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio. We predict that if and when such an evolutionary history of the mantle is reconstructed, it will bear no resemblance to that shown in Fig. 1 of Brown *et al.*

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WE thank Alexander and Schwartzman (AS)<sup>1</sup> for bringing to light a significant error in data point 18 (Table 1) of our paper<sup>2</sup>. Because of a misinterpretation of a verbal communication from Mueller, we omitted sample 30 in our regression of the data from Baadsgaard and Mueller<sup>3</sup>. We confirm that there is no reason for omitting this sample, and that the data indicate that the non-radiogenic argon present cannot be distinguished from atmospheric contamination. We concur with Alexander and Schwartzman, however, on little else, and consider as still valid the four principal points summarised at the end of our paper<sup>2</sup>: first, the initial  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios of mantle-derived igneous rocks have increased steadily over the past 800 Myr. Second, the present day  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios in the upper mantle and atmosphere are very similar. Third, the majority of argon at present in the atmosphere has been derived from the mantle rather than the crust, and fourth, initial  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios from igneous rocks are potentially more sensitive petrogenetic indicators than strontium or lead isotopes.

Alexander and Schwartzman are unable to reproduce similar ages in their Table 1 for two reasons: first, they consider all  $^{36}\text{Ar}$  to be atmospheric contamination and second, they use a different half-life. We have used the half-life value recommended by Beckinsale and Gale<sup>4</sup>. Thus, as we approach the matter differently, we are likewise unable to reproduce in detail their Table 1 and Fig. 2.

With regard to point 17 of our paper, Argyll biotites, AS prefer to use the intercept of 3 analyses on one sample as the best estimate of the initial ratio. Since line blank is still a major problem in argon isochron analyses, and this sample was not blank corrected, the regression of these

three analyses will give an intercept corresponding to the blank  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio which is atmospheric in origin. Therefore it is not valid for AS to plot this point (17b) on their Fig. 2. Further an age of  $389 \pm 1$  Myr, as suggested by this regression, is too young, since the samples predate, geologically, the granites and lavas of the region which are dated independently by Rb-Sr isochrons at  $425 \pm 5$  Myr ( $t_{1/2} = 5.00 \times 10^{10}$  yr).

Points 3 to 7 refer to McDougall *et al.*'s data<sup>5</sup>. We do not agree that the evidence for the young age of Rangitoto is unequivocal. The samples taken for the radiocarbon ages were covered by tuffs and a basalt flow which represent the latest activity on the volcano. The volcano could have been built up episodically over the past 0.5 Myr. Once again we are only suggesting an alternative interpretation.

A correction of Kistler's data<sup>5</sup> to allow for the spike contribution yields a  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio even lower than we had thought. AS cite petrographic descriptions of the diorite as "evidence of alteration". The cited descriptions, however, indicate the quite pristine nature of these rocks<sup>7</sup>. The mantling of clinopyroxene by hornblende is a normal phase relationship developed during the crystallisation of diorites.

Krummenacher<sup>7</sup> only reported three samples in his Appendix 2 as having a "true"  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio; these are the values we report as our point 1. The rest of his data are not tabulated or corrected in this fashion. Rather than ignoring the possibility of mass fractionation, we agree that it is an important factor which should not be forgotten when analysing young volcanic rocks. What we did say in our paper was that "the possibility of diffusion and fractionation of atmospheric argon into the cooling lava does not seem realistic to us". Krummenacher did not "demonstrate", he merely suggested that fractionation could be the cause of some low initial ratios.

For several of the groups of data AS have repeated the various explanations offered by the investigators (for example the quote by McDougall *et al.*<sup>5</sup> regarding the Auckland data). We have not ignored the explanations given by others for apparently sub-atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios, but have tried to offer a single alternative interpretation of the data, given the possibility that these low initial ratios do exist. This possibility has been considerably strengthened by the recent work of Mellor and Mussett<sup>8</sup>. The explanations of others, while perhaps equally valid, do not negate our interpretations. If one can explain low ratios in mantle derived rocks, any higher-than-atmospheric ratios present no problem; they may be explained by higher than average potassium concentration in the source region, extensive outgassing of the source region or xenolithic contamination of the rising magma. For this reason alone, one could justify the selection of low ratios for our model as they represent the closest approach to primordial argon from which mantle argon evolved. This justification is the same as that used in models of the mantle growth of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios, even though the mechanics and complexities of the models are obviously different. It is not necessary to evolve elaborate mixing or fractionation models to explain low ratios in mantle-derived rocks, and these low ratios are no more 'anomalous' than the high ratios commonly thought of as containing 'excess argon'.

AS perceptively point out that the line shown in our Fig. 1 extrapolates to  $-666$  at 4,500 Myr. Because of this, they claim the line is a physical impossibility, but such a conclusion is hardly valid. The line was intended to be nothing more than an indication of a first-order variation over the time indicated. The rate of growth of  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios in the mantle should not be linear, as it is strongly affected by the outgassing history. If Alexander and Schwartzman applied the same reasoning to their own Fig. 2, they might conclude that the atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio 4,500 Myr ago was 295.5.

They make reference to Shafiqullah and Damon's<sup>9</sup> caution against using argon isochrons, this is all very well if they concur with the main postulate of that paper, all  $^{36}\text{Ar}$  measured in an analysis comes from the atmosphere. This postulate was discussed in our paper and rejected in favour of the assumption that a substantial proportion of the  $^{36}\text{Ar}$  measured came from the sample itself. In their very next paragraph AS take the opposite stance and claim that the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios measured in ocean floor pillow basalts represent the  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio of the mantle source region.

Perhaps we should have explained more fully our estimate of the amount of  $^{40}\text{Ar}$  in the Earth's crust. For the sake of brevity and not anticipating such naiveté from interested readers we did not mention that the value of 4,500 Myr was selected because it is a defensible limiting condition and gives a maximum crustal  $^{40}\text{Ar}$  value. We would be quite content with a smaller value because it is our contention that the crustal contribution to the atmosphere has been minor relative to the mantle contribution.

AS's statement that "the young average age of the continental crust, about 1,100 Myr, implies that most of the argon is degassed during uplift, weathering, metamorphism (radiogenic  $^{40}\text{Ar}$  is found in natural gases), continental growth, and recycling in subduction zones" is fraught with fallacies.

Any such implication would require a comparison of the real age of all crustal material with the respective K-Ar ages. Such a comparison cannot be made, however, for the relevant K-Ar data are minuscule. The only relevant data are from unmetamorphosed rocks, where, in fact, the K-Ar ages are in good agreement with the real ages. The K-Ar data available for the vast metamorphic terranes of the crust are almost entirely from high potassium mineral phases, such as biotite. These minerals are hardly representative of continental crust. There are so little data from metamorphic rocks on the low-potassium phases (for example, plagioclase, pyroxene, and amphiboles), where 'excess argon' is common, that it is premature to attempt to evaluate the average K-Ar age of the continental crust.

Argon is not degassed during uplift. We are startled that Alexander and Schwartzman think otherwise. The evidence is too voluminous and familiar to geologists to cite here, but a large number of studies have shown that uplift results in argon retention.

Some argon is released to the atmosphere by weathering, but most of this would be taken into account in our consideration of sedimentary rocks. In fact it is possible that the retention of Ar by detrital material has led us to overestimate the significance of this surface process.

Metamorphism takes place at depth and the argon becomes mobile, at least on the whole-rock scale, but it cannot pass upwards through the cold capping rocks, except, perhaps along major fissures. If this were not the case, then the identification of excess argon in low potassium phases in metamorphic rocks would be extremely rare.

The fact that "radiogenic  $^{40}\text{Ar}$  is found in natural gases" has no bearing on this matter. Alexander and Schwartzman refer to work done by Moorbath *et al.* in Iceland. It is perplexing to wonder what relationship they think this has to outgassing and metamorphism of continental crust.

There is absolutely no evidence that argon is outgassed during "continental growth and recycling in subduction zones". On the contrary, isotopic studies indicate the insignificant role of older continental material in the generation of new continental crust and also that there is either no recycling, or it is a process which has escaped detection.

One of the major differences between our models and the thesis of Alexander and Schwartzman is that they consider the whole Earth as being equally capable of degassing,

whereas we consider the upper mantle and the crust as separate systems with only the upper mantle having the capability for major outgassing through volcanism.

We believe that low initial  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios do exist in mantle-derived rocks and that these ratios reflect a mantle, or region of the mantle, which had a  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio of  $<295.5$  when the rocks formed.

If the mantle does contain a source of argon which, not long ago, had a  $^{40}\text{Ar}/^{36}\text{Ar}$  ratio less than the present-day atmospheric value, then it is relatively easy to explain any higher initial ratios observed. The real difficulty is encountered when one attempts to explain the derivation of low ratios from a mantle with high  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios. Alexander and Schwartzman recognise this difficulty, but can circumvent it by not accepting the existence of initial  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios less than the present-day atmosphere. Our interpretation has its own difficulties, but none so severe as indicated by AS. We have tried to account for less than atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios in the mantle. The model promoted by Alexander and Schwartzman cannot account for, and in fact denies, the possibility of such ratios. It must distress AS somewhat to know that  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios significantly less than atmospheric have been directly measured from the Golovnin volcano (Kurile Islands) on two separate occasions by different researchers<sup>10,11</sup>. Also there is now an example of an extremely low  $^{40}\text{Ar}/^{36}\text{Ar}$  initial ratio in the 625-Myr-old Franklin diabase dykes<sup>12</sup>, and data from the 60-Myr Antrim basalts<sup>8</sup> indicate lower than atmospheric  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios in agreement with our model.

The real contention thus lies in whether or not mantle-derived rocks can contain  $^{40}\text{Ar}/^{36}\text{Ar}$  ratios of less than that of the present-day atmosphere. Future research will resolve this contention.

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## Temperature dependence of the anomalous specific heat of glasses and amorphous solids

THE low temperature specific heat of glasses and amorphous solids is characterised by an anomalous linear variation with temperature<sup>1–6</sup>. Of the proposed theoretical explanations (reviewed by Leadbetter<sup>7</sup>), the most favoured attributes this anomaly to localised two-level systems<sup>8–9</sup> or to low frequency vibrations of single atoms or molecules trapped in cavities<sup>10,11</sup>. It is difficult, however, to conceive of a level-splitting mechanism that would be sufficiently general to explain the apparent universality of the anomaly. Moreover, several impurities that could have produced two-level systems have now been eliminated as possible candidates<sup>12,13</sup>. An explanation based on low frequency vibrations of atoms or molecules in cavities rests on two *ad hoc* assumptions about the size and distribution of the cavities, and Stephens *et al.*<sup>5</sup> argue that to account for the

experimental results, these assumptions seem to be physically implausible.

Experimental evidence implies that the amorphous state may be thought of simply as the crystalline state saturated with the most favoured dislocations<sup>14</sup>. This accounts for the densities of the crystalline and the amorphous state being very similar. Various aspects of the mechanical behaviour of metallic glasses have been discussed fruitfully in terms of dislocations and their movements<sup>15–19</sup>, and we show here that this also accounts for the enhanced specific heat of glasses and for their anomalous linear temperature dependence.

Crystal defects can affect the specific heat of a solid by changes in the distribution of vibrational frequencies<sup>20</sup>. Further, extended defects or dislocations can produce low frequency vibrations which are effective at low temperatures. At sufficiently low temperatures ( $\sim 1$  K) it is not unreasonable to expect that dislocations will be pinned either by impurities or at their crossing points. Granato<sup>20</sup> analysed the pinned-dislocation contribution to the specific heat per mole,  $C_v$ , and found that at low temperature

$$C_v = \frac{p\pi^2}{3} \frac{\Lambda a^2}{Z} \frac{Nk}{\theta} T \quad (1)$$

where  $p = v_o/C$  ( $v_o$  is the velocity of sound in the perfect lattice and  $C$  is given by the relation  $C = (G/\rho)^{1/2}$  where  $G$  is the shear modulus and  $\rho$  the density),  $\Lambda$  is the dislocation density,  $a$  the lattice constant,  $Z$  the number of atoms per unit cell,  $N$  the number of atoms per mole, and  $\theta$  the Debye temperature. Thus, for a given solid, the contribution of the vibrations of pinned dislocations to the specific heat is of the observed form. It is interesting to note that equation (1) depends on dislocations only through their density, and that the distribution of dislocation lengths is irrelevant. One also notes that neither the Burgers vector nor its distribution enters the expression. At  $\sim 1$  K the dislocation contribution to the specific heat may be of the same order of magnitude as that of the lattice. For heavily cold-worked aluminium at 1 K this contribution was found to be 20% of the total specific heat and a larger fraction at lower temperatures<sup>20</sup>.

In seeking to obtain quantitative corroboration of these ideas one must contend with the fact that experimental data for glasses invariably refer to multi-element compositions, so average values must be inserted in equation (1). Golding, Bagley and Hsu<sup>21</sup> find that for  $\text{Pd}_{78}\text{Si}_{16}\text{Cu}_6$  the anomalous linear region gives  $C_v/T = 1.3 \times 10^4$  erg mol<sup>-1</sup> K<sup>-2</sup>. Using the weighted averages  $a = 3.8 \times 10^{-8}$  cm,  $\rho = 10.3$  g cm<sup>-3</sup>,  $G = 8 \times 10^{11}$  dyne cm<sup>-2</sup>,  $\theta = 344$  K,  $Z = 4$ , and noting (with Granato<sup>20</sup>) that  $v_o = ak\theta/\pi\hbar$ , one finds from equation (1) that  $C_v/T = 5.6 \times 10^{-10} \Lambda$  erg mole<sup>-1</sup> K<sup>-2</sup>. The saturation condition corresponds to the situation in which adjacent dislocation cores are contiguous, which implies that  $\Lambda = (r_c^2/3)/2$ , where  $r_c$  is the core radius. For a typical metal, such as copper, it has been found that the core radius is  $\sim 3.6$  Burgers vectors<sup>22</sup>. The most likely shear dislocation in close-packed structures is the Shockley partial with a Burgers vector of  $0.41a$ . Thus saturation corresponds to  $\Lambda = 9.2 \times 10^{13}$  cm<sup>-2</sup>, giving finally  $C_v/T = 5.2 \times 10^4$  erg mol<sup>-1</sup> K<sup>-2</sup>. In view of the difficulty involved with using average values the agreement with the result of Golding *et al.* is quite good.

Because core radii are invariably a few Å, the saturation dislocation density is roughly constant so it is not surprising that the anomalous specific heat contribution for different substances does not vary greatly<sup>2,6</sup>. Further, the scattering of phonons by dislocations implies that the thermal conductivity, if this type of scattering is dominant, would be  $\propto T^2$ . It is experimentally observed that the temperature exponent of the thermal conductivity for non-crystalline solids varies between 1.8 and 2.0<sup>2,6,23</sup>. (It should be noted that although Lasjaunias *et al.*<sup>23</sup> found that the thermal conductivity of vitreous  $\text{B}_2\text{O}_3$  between 0.1 and 1.3 K varies as  $T^{1.9}$  the excess specific heat contribution seemed to vary as  $T^{1.46}$  between 60 mK and 0.6 K. The reason for this behaviour is not clear to us.) In the limit as  $T \rightarrow 0$  equation (1)

is invalid, and Granato<sup>20</sup> gives an expression

$$C_v = \frac{\Lambda}{L} \frac{a^3}{Z} Nk \left( \frac{\hbar \omega_0}{kT} \right)^2 \exp(-\hbar \omega_0/kT) \quad (2)$$

which goes exponentially to zero at very low temperatures ( $\omega_0$  corresponding to the lowest vibrational mode). This indicates a method of testing these ideas.

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## Cementite structure for iron sulphide, Fe<sub>3</sub>S

IN our studies on the growth of iron sulphides in an inert gas, we synthesised a new crystal form of iron sulphide having a cementite-type structure. The method was the following: a fine powder of synthetic troilite (Fe<sub>1.0</sub>S) was flash evaporated in 99.99% pure argon gas at 100 mmHg, and passed into a vacuum chamber initially at a pressure of 10<sup>-6</sup> mmHg. A thin film was obtained on some water-cooled microgrids 10 mm from the evaporation source (1,900 °C) and was examined in a Hitachi HU-11D electron microscope equipped with the model HXA-1 electron probe microanalyser (with mica as dispersing crystal).

The intensities and lattice spacings as observed and calculated are listed in Table 1. The pattern can be indexed on the basis of an orthorhombic unit cell with  $a=4.56$  Å,  $b=5.09$  Å,  $c=6.70$  Å.

Cementite<sup>1</sup>, Fe<sub>3</sub>C, space group Pbnm, has an orthorhombic unit cell with  $a=4.523$  Å,  $b=5.088$  Å and  $c=6.743$  Å. On the other hand, vanadium subsulphide<sup>2</sup> β-V<sub>3</sub>S is known to have a tetragonal unit cell (P<sub>4</sub>/nbc) with  $a=9.381$  Å and  $c=4.663$  Å. From the better agreement with the lattice constants of the former, the new crystal probably has the cementite structure. The electron diffraction data for the iron sulphide yield no further information, since there are too many overlapping and coinciding peaks.

The chemical composition of the thin film was established roughly by X-ray emission spectra, using 2C-type pyrrhotite film (FeS) as a standard. A film was prepared by the flash evaporation of the same starting material (Fe<sub>1.0</sub>S) in argon at 35 mmHg, and its chemical composition was determined by the presence of the  $d(100)=5.16$  Å line of the 2C-type superstructure. The intensity measurements of the Kα lines of Fe and S reveal that the new phase is very much richer in iron than sulphur, and the composition falls between Fe<sub>2.5</sub>S and Fe<sub>2.8</sub>S.

Table 1 Electron diffraction data for the new cementite-type iron sulphide

<i>hkl</i>	<i>d</i> <sub>obs</sub>	<i>d</i> <sub>calc</sub>	<i>I</i> <sub>obs</sub>	<i>hkl</i>	<i>d</i> <sub>obs</sub>	<i>d</i> <sub>calc</sub>	<i>I</i> <sub>obs</sub>	<i>hkl</i>	<i>d</i> <sub>obs</sub>	<i>d</i> <sub>calc</sub>	<i>I</i> <sub>obs</sub>
001		6.70		203		1.60		232		1.26	
010		5.09		014	1.59	1.59	16	303	1.25	1.26	8
100		4.56		130		1.59		041		1.25	
011		4.05		123		1.57		115		1.25	
101		3.77		104		1.57		140		1.23	
110	3.39	3.40	10	131		1.55		313	1.22	1.22	19
002		3.35		213	1.53	1.52	9	322		1.22	
111		3.03		300		1.52		141		1.21	
012		2.80		222		1.51		224		1.19	
102		2.70		032	1.50	1.51	1	034		1.19	
020	2.53	2.54	5	114		1.50		042		1.19	
112		2.38		301		1.48		025		1.19	
021	2.39	2.38	8	310		1.46		233	1.17	1.16	10
200	2.28	2.28	15	132		1.44		205		1.16	
003	2.22	2.23	17	311		1.42		134		1.15	
120		2.22		024	1.40	1.40	3	142		1.15	
201		2.16		302		1.38		125		1.15	
121	2.11	2.11	36	230		1.36		400	1.14	1.14	25
210	2.08	2.08	53	033		1.35		330		1.13	
013		2.04		223		1.35		215		1.13	
022	2.04	2.03	100	204		1.35		323		1.13	
103		2.00		005	1.34	1.34	12	304		1.13	
211	1.99	1.99	39	124		1.34		401		1.12	
202		1.88		312		1.34		006		1.12	
113	1.87	1.87	25	231		1.33		331		1.12	
122	1.84	1.85	18	320		1.30		410		1.11	
212	1.76	1.77	15	214		1.30		240	1.11	1.11	21
030		1.70		015	1.29	1.30	8	043		1.11	
220	1.69	1.70	12	133		1.30		314		1.10	
023		1.68		105		1.29		411		1.10	
004		1.67		321		1.28		241		1.10	
221		1.65		040		1.27		016		1.09	
031	1.64	1.64	4					106		1.08	

$$a = 4.56 \text{ Å}, b = 5.09 \text{ Å}, c = 6.70 \text{ Å}.$$



It is postulated that the film-like deposits form at 100 mmHg because of the high density of vapour in the narrow condensation region which comprises different supersaturation zones. This is confirmed by the fact that thin films composed of 2C-type pyrrhotite ( $\text{FeS}$ ), marcasite and pyrite ( $\text{FeS}_2$ ) were obtained near the evaporation source except for a few regions where the iron-rich sulphide was the single phase.

There are two restrictions on the conditions of film formation of the iron sulphide in this experiment: first, the only position at which the thin film could be obtained was at a distance of 7–15 mm from the evaporation source. Further away, the subsequent growth leads to the formation of iron sulphide smoke particles, until they aggregate into a chain of composition  $\text{Fe}_{1-x}\text{S}$ . Second, at lower pressures, 5 and 35 mmHg, the thin film obtained at the same position was not the cementite-type iron sulphide, but only 2C-type pyrrhotite used above as the standard sample. Further details will be published elsewhere.

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## Growth rates of freely falling ice crystals

THE only available set of laboratory measurements of mass growth rates of freely falling ice crystals, as a function of both time and temperature, used growth times < 50 s (ref. 1). We report here results of laboratory measurements on freely falling ice crystals (average mass,  $\bar{m}$ ) grown in simulated cloud conditions, as a function of temperature for growth times of  $60 \pm 3$  and  $100 \pm 5$  s, and from these we have calculated the corresponding mass growth rates.

We wished to simulate the growth processes occurring in natural clouds, and therefore the measured ice crystals were produced in, and then grew while falling freely

Fig. 1 Average mass of freely falling ice crystals as a function of temperature for growth times of 1,  $60 \pm 3$  s and 2,  $100 \pm 5$  s.

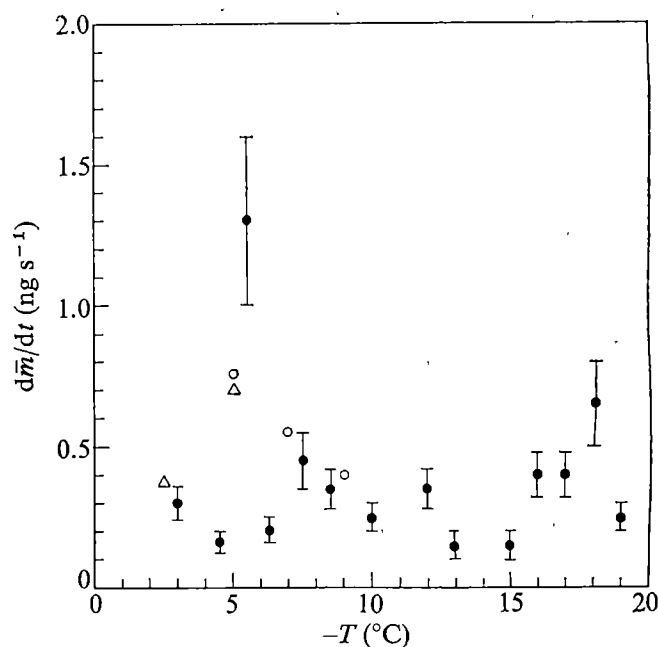
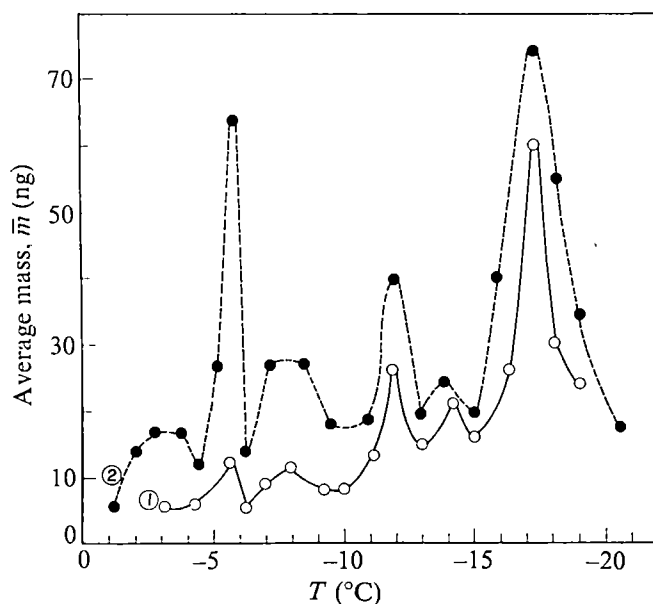


Fig. 2 Average mass growth rate of freely falling ice crystals as a function of temperature in the time range 60–100 s. calculated from: this work (●); Mason<sup>9</sup> (△) and Ryan *et al.*<sup>10</sup> (○)

through, fogs of supercooled water droplets with size spectra and concentrations similar to those of many rain clouds. The ratio of droplets to ice crystals was always > 8 : 1.

Ice growth was initiated by the rotation of a metal rod, cooled with liquid air, which locally seeded the supercooled fog with tiny ice crystals. The settling ice crystals were sampled by collecting them on a cooled glass slide coated by a layer of highly viscous silicone oil (DC 200/1,000). Each of the samples comprised ~ 150 crystals.

The average mass values of the samples were calculated from the measured diameters of the water droplets resulting from melting the collected ice crystals. Details of the experimental system and procedure are described elsewhere<sup>2</sup>.

Figure 1 shows the temperature-dependent average mass of ice crystals, sampled at  $60 \pm 3$  and  $100 \pm 5$  s after seeding, and Fig. 2 shows their calculated temperature-dependent average mass growth rate in the time range 60–100 s.

It is obvious from Fig. 2 that, first, there are peaks in the growth rate at ~ -5.5 and -18 °C, as have been found previously<sup>1,3,4</sup>; second, the maximum at about -5.5 °C is similar to the behaviour of the basal linear growth rate<sup>5</sup>, basal mean migration distance<sup>6</sup> and basal step propagation velocity<sup>7,8</sup>; and third, the few growth rates for the range 60–100 s, calculated from experiments by Mason<sup>9</sup> and Ryan *et al.*<sup>10</sup> are in good agreement with our results.

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## 'Braille' reading by a blind volunteer by visual cortex stimulation

THE possibility of developing functional visual prostheses was supported by the pioneering case of Brindley and Lewin<sup>1,2</sup> and confirmed by our subsequent report on two other blind patients<sup>3</sup>.

Although much work remains to be done on engineering design<sup>4,5</sup> and on minimising the effects of continuous chronic electrical stimulation of the brain<sup>6,7</sup>, the most fundamental question remains the demonstration of useful information transfer by cortical stimulation.

We therefore report preliminary experiments in which a blind volunteer was, without practice, able to read 'braille' transmitted by cortical phosphenes at 30 letters min<sup>-1</sup> (much faster than he could read tactile braille). This patient was also able to use a TV camera to detect horizontal and vertical lines. These experiments with dynamically changing pattern presentation are the first demonstration of potentially useful information transfer by cortical stimulation.

Similar attempts to transmit 'cortical braille' to Brindley's second patient<sup>8</sup> were hampered by large, anomalous phosphenes which limited reading to 8.5 characters min<sup>-1</sup>, much slower than the patient could read tactile braille. It should, however, be emphasised that as yet we regard 'cortical braille' primarily as a technique to begin investigation of dynamic pattern presentation, rather than as a basis for clinically useful prostheses.

Our previous experiments<sup>3,9</sup> used temporary electrode arrays removed a few days postoperatively, but the time limitations of this approach are a serious drawback. Consequently, we designed a chronic implant (Fig. 1) by modifying our ribbon cable electrodes to connect with a special percutaneous pedestal<sup>10</sup> developed in the course of our parallel programme on auditory prostheses.

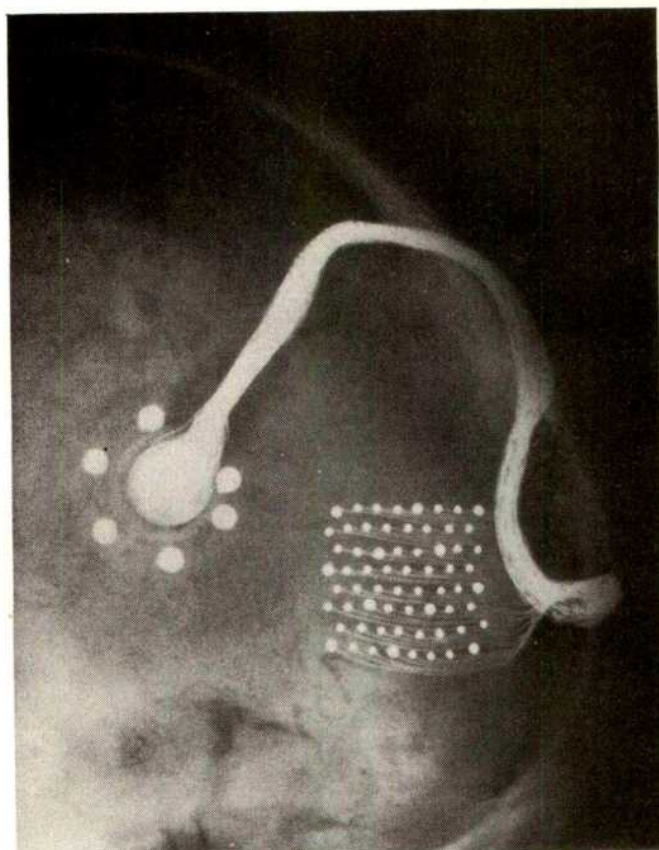
The volunteer, a 33-yr-old man, was blinded ten years ago by gunshot. He had been associated with the project for several years and his fully informed consent had been obtained<sup>3</sup>. Surgical techniques and postoperative instrumentation were similar to those described earlier<sup>3</sup>, with the addition of a camera based on a 100×100 charge-coupled phototransistor array interfaced with the computer system.

Most stimulation has been conducted with 0.1–1.0-s trains of constant current, symmetrical, biphasic square pulses, coupled through 0.47  $\mu$ F series capacitors, typically of zero to peak amplitude 0.5–3.0 mA, of 0.25/0.25 ms duration (–/+), repeated at 50 Hz. All simultaneous stimulation of multiple electrodes involved individual amplitude adjustments to a constant multiple (typically 1.25) of threshold, and all trains were interlaced typically with 1.0-ms intervals between pulses delivered to different electrodes in numerical order.

Sixty of the 64 electrodes have continuous connecting wires. All produce point phosphenes (some are multiples) at thresholds ranging from 0.8–4.0 mA zero to peak (1.7 mA average with a median of 1.4). These thresholds fluctuated during the postoperative consolidation period, stabilising during the fourth week. They do not seem affected by the limited, intermittent, stimulation involved in our experiments to date. As expected<sup>9</sup> there are no significant differences in phosphene appearance or thresholds based on electrode area.

Electrode 'impedances' are complex and nonlinear. For 0.25/0.25 ms duration (–/+) pulses, 5.2–21.5 V peak to peak (average 8.5; median 8.4) are necessary to force 3.0 mA peak to peak. There is no clear correlation of 'impedance' with either threshold or electrode area.

A detailed map (Fig. 2) of the relative position of each phosphene in the visual field was prepared under computer control<sup>3</sup> with adjustments for absolute distances between



**Fig. 1** Array of 64 platinum electrodes in a Teflon matrix. They are hexagonally packed on nominal 3-mm centres, in the subdural space on the right medial occipital cortex. 12 electrodes are 2 mm<sup>2</sup> in area and the remainder 1 mm<sup>2</sup>. In this X ray they are numbered in horizontal rows (number 1 in the upper right, number 8 in the upper left, number 57 in the lower right, and number 64 in the lower left). The integral platinum connecting wires are individually insulated with Teflon and form a small cable which passes through the skull and folds beneath temporo-occipital periosteum. The wires terminate in a special high density connector inserted in a percutaneous Pyrolite carbon pedestal, attached to the cranial vault with special bone screws.

phosphenes based on a manually prepared map.

As previously reported<sup>3</sup> simple letters and patterns can be recognised easily, although this is hampered by interactions which often occur between phosphenes which are neighbours in visual space (regardless of the position of the corresponding electrodes in the array). The number and spatial distribution of phosphenes is inadequate for presentation of 26 ordinary letters. Consequently, as indicated in Fig. 2, six non-interacting phosphenes were selected to form a 'braille cell'.

Using randomised alphabets, presented as 0.5-s trains of cortical stimulation at intervals controlled by the patient's response, he averaged 19 (17–20) correct responses out of 26 letters (73%) over an average period of 47 (44–56) s. Changing the method of presentation slightly so that each letter was represented by a 0.5-s train at regular 2.0-s intervals, the patient averaged 85% correct responses in three trials with similar random alphabets.

The results with 'cortical braille' contrast sharply with similar experiments using tactile braille scanned at the same rate. Random alphabets were punched on file cards to optimise tactile sensation, and the patient permitted to examine each letter for 2 s. A tone indicated a shift to the next letter, and in these conditions the patient averaged only 28% (23–35%) correct responses in each of three trials.

We then changed to sentence presentation using 'cortical braille' at a rate of 30 characters min<sup>-1</sup> (0.5 s trains delivered every 2 s, with a 4-s pause between words). The initial presentations (selected from a braille reading test) and responses were as follows: (1) "When the crow went into"

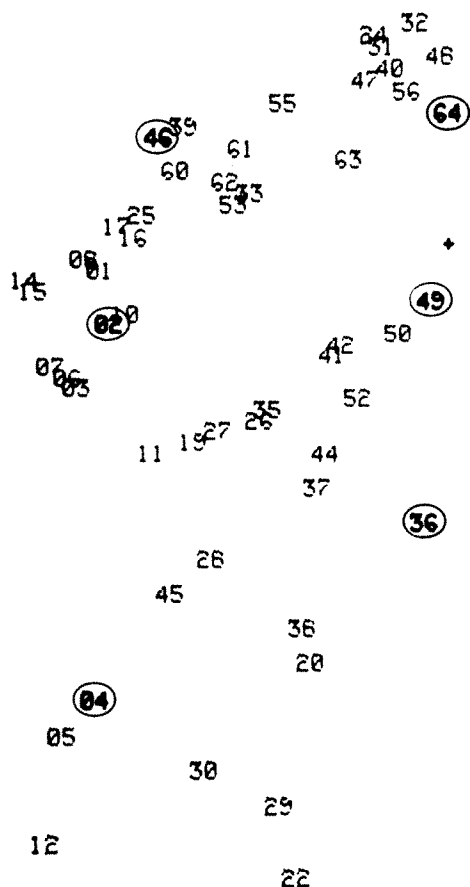


(54 s) read as "... crow went n to". (2) "He had a cat and ball" (52 s) read as "He his cat and ball". (In this case he actually spelled the word "has" properly out loud but called it "his".)

The patient who has a high-school education is a very poor braille reader, averaging about 1 word min<sup>-1</sup> on high school level, grade II (contracted) braille; consequently, he rarely uses tactile braille. He believes, and we concur, that he will become considerably faster at 'cortical braille' with practice, particularly when he can control the rate of presentation through a modified tablet now being interfaced with the computer. Preliminary experiments with kinaesthetic feedback were conducted using the IC camera interfaced with the computer system (0.5-s trains with a maximum of 8 electrodes per frame). Although complicated by phosphene interaction, the patient could distinguish horizontal and vertical strips of 1 inch wide white tape on a black background, and further experiments are now in progress.

Experiments with 'cortical braille' and the camera emphasise the need for interframe delays, particularly when using very short trains (for example, 125-ms trains repeated at 250-ms intervals). At frame rates faster than 4 s<sup>-1</sup>, presentations tend to blur, regardless of the patient's ability to recognise and retain individual characters. From the patient's description, we believe that such blurring results from short duration phosphene persistence, compounded

**Fig. 2** Cathode ray tube map of phosphene positions in visual space. The phosphene produced by electrode number 22 is about 22° from the fixation point marked by (+). As expected<sup>1-3,9</sup> there are many discrepancies from the classical retinotopic map. For example, electrodes far from the occipital pole produce central phosphenes. Adjacent electrodes (for example numbers 30, 31 and 32) may produce widely spaced phosphenes, perhaps due to intervening fissures. Adjacent phosphenes, however, produced by distant electrodes (for example numbers 1 and 16) are more difficult to explain. The circled bold numbers indicate the 6 phosphenes forming the 'braille cell' (electrodes number 46, 2, 4, 64, 49 and 36).



by the intrinsic phosphene flicker. Further experiments on both of these phenomena are also in progress.

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## Ethylene-induced volatile inhibitors causing soil fungistasis

THE phenomenon of soil fungistasis<sup>1-3</sup>, a term introduced by Dobbs and Hinson<sup>1</sup> to describe the failure of almost any fungus propagule to germinate in the upper layer of non-amended natural or cultivated soil, has been attributed to volatile inhibitors. Evidence implicating volatile unsaturated hydrocarbons was first obtained from the observation that when soil blocks are incubated in closed containers over either silver nitrate or mercuric perchlorate solutions, they exhibit reduced fungistatic activity against conidia of *Arthrobotrys oligospora*<sup>2</sup>.

To test the hypothesis that ethylene is the main inhibitor involved in soil fungistasis<sup>3</sup>, nutrient-free water agar disks 2 mm thick (Special Agar Noble, Difco), carrying conidia of *A. oligospora* were incubated at 22 °C in atmospheres containing up to 50% (v/v) ethylene (Ethylene CP Calibration Mixture, Commonwealth Industrial Gases). Contrary to what should have been expected if ethylene had any fungistatic activity, conidia germination in all cases proceeded unrestricted, and equalled that of the control series in ethylene-free atmospheres. The use of nutrient-free agar renders unlikely the argument that results obtained in pure culture cannot be extrapolated to unsterilised soil because the fungistatic action can be overridden by nutrients<sup>4</sup>. This

becomes even more apparent in view of the following tests. Using a modification of the Romine and Baker soil emanation technique<sup>7</sup>, agar disks, similar to the ones used in the ethylene bioassays, were laid in disposable Petri dish halves (3.5 cm diameter). These were placed inverted on the surface of 100-g soil samples. A glass vial (10 ml), containing a folded piece of glass-fibre paper and either silver nitrate solution (2.5% w/v) or water, was included in the headspace of soil in each Petri dish. The soils, in this and subsequent experiments, were collected on the day of use and sieved to 0.2 cm while still moist. After 3 d incubation at 23 °C, the fungistatic activity of the agar disks was assessed by seeding, on their lower surface, conidia of either *Penicillium chrysogenum* or *P. italicum* and counting the percentage germination after 18 h further incubation in the same conditions. The experiment was repeated a number of times in triplicate using originally Irish (clay loam, pH 6.6, University College, Dublin) and subsequently Tasmanian (sandy clay loam, pH 5.5, Hobart area) soils. On all occasions the results were of comparable order and showed that the agar disks of the silver nitrate series were less fungistatic compared with controls. Typical results are presented in Table 1. Kouyeas, in the Agricultural College of Athens, using a similar experimental arrangement and four soils from southern Greece (pH 7.6–7.9), obtained comparable results with eight different fungal species<sup>8</sup>.

The fact that agar disks acquire fungistatic properties when exposed to volatile emanations from soil, but not when incubated in the presence of ethylene, leaves no doubt as to the ineffectiveness of the latter as a spore germination inhibitor. Examination of the electronic spectra of water condensates collected from soil headspace revealed, however, that ethylene-like substances were present in the soil gaseous emanations.

In these experiments, an all-glass cold finger condenser, with a small vial suspended from its lower end, was suspended over 500-g soil samples held in conical flasks (2 l). During the experiment the soil temperature was kept at 25 °C whereas that of the water circulating in the condenser was about 10 °C. An incubation period of about 24 h was usually sufficient for the collection of 5–10 ml water condensate in the receiver vial, beneath the condenser.

Water condensates from both Irish and Tasmanian soils had a greater  $A_{196}$  than distilled water. By adding dropwise a freshly prepared bromine water solution into the sample cell only, an absorption band emerged in the region of 165 nm, the intensity of which increased with further addition of bromine.

Trimethylamine or formic acid ( $1 \text{ mg l}^{-1}$ ) could account for the  $A_{196}$ , but neither showed any response to bromine treatment; and when they were neutralised both failed to inhibit spore germination. Water saturated with ethylene or solutions of other compounds containing an ethylene double bond, when treated with bromine, gave an absorption curve similar to the one that was given by soil water condensates under the same treatment (Fig. 1), indicating the presence of ethylene-like substances among the soil gaseous emanations.

Since ethylene itself has no inhibitory effect on spore germination, however, Smith's observation, that soil fungistasis is enhanced in the presence of ethylene<sup>3,9</sup>, leads to the alternative hypothesis that ethylene might trigger or accele-

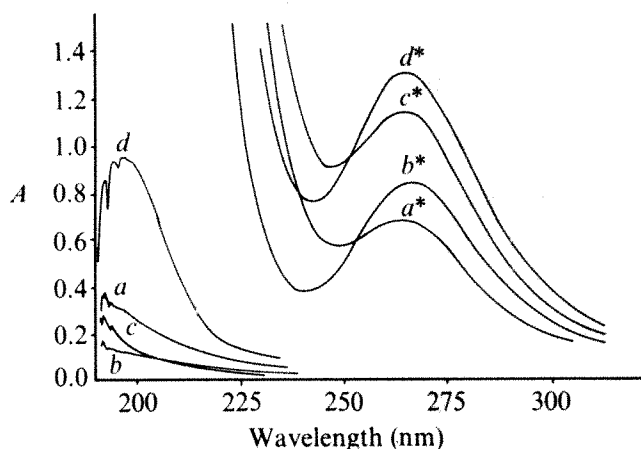


Fig. 1 Absorption spectra of water condensates of soil headspace (a), water saturated with ethylene (b), allyl alcohol (c) and acrylic acid (d).  $a^*$ – $d^*$ , Same as above after treatment with bromine.

rate the process by which the inhibitor(s) is produced. Water condensates were collected, as described above, from soil samples (1 kg fresh weight) which had been pre-incubated for 3 d in atmospheres of air only or air supplemented with 1% ethylene. After collection, the water condensates were transferred to glass vials, which were sealed with rubber septa and placed in a 60 °C water bath. Gas samples (2 ml) were withdrawn from the headspace of each vial and analysed in a Pye Unicam 104 Gas Chromatograph with a  $1.60 \times 0.003 \text{ m}$  column packed with 5% Carbowax 20M on Gas Chrom Q (80/100 mesh), 60 °C,  $\text{N}_2$  carrier gas and  $\text{H}_2$  flame ionisation. Condensates from the ethylene-treated soil yielded, apart from a peak attributable to ethylene, several others with poor resolution and one with retention time similar to allyl alcohol (approximately 5.5 min). Characteristically, no peak was registered when samples of water condensates from air-treated soil were used. Incubation of soil in presence of ethylene thus resulted in the production of other volatile substances; although they are awaiting identification and assessment of their significance, formation of allyl alcohol alone may account for the enhancement of fungistatic activity in soils treated with ethylene as the following microdiffusion germination tests indicate.

Although acrylic acid was not detected, it was included in the bioassays because of its close similarity to allyl alcohol, the fact that Shmuk (cited in ref. 10) had included this acid in a list of organic substances isolated from soil by numerous investigators, and its identification with the factor responsible for the antibacterial properties of phytoplankton and seaweeds<sup>11–14</sup>.

Bioassays were carried out using a microdiffusion system designed to simulate the conditions of the spore germination tests on soil blocks<sup>2</sup> and those of the agar disk technique above. Films (1 mm thick) of nutrient-free agar carrying conidia of *A. oligospora* were incubated over solutions of either allyl alcohol or acrylic acid in containers similar to Conway's microdiffusion dishes (3.4 cm diameter, 2 cm deep; central compartment, 1.4 cm diameter, 1 cm deep). Aliquots (5 ml) of the compound under test were placed in the outer compartment of the dishes whereas the inner compartment received 1 ml silver nitrate solution (2% w/v) and a small pleated piece of glass-fibre paper to increase the absorbing capacity of the solution.

Table 2 shows that the diffusing vapours from both allyl alcohol and acrylic acid solutions were fungistatic and that the presence of silver nitrate resulted in a dramatic reduction of their inhibitory effect. The fact that allyl alcohol seems more effective (quantitatively) than acrylic acid

Table 1 Percentage spore germination on nutrient-free agar disks pre-exposed to volatile emanations from soil, in the presence or absence of silver nitrate solution

Soils	Test organisms	Silver nitrate solution	
		Present	Absent
Irish	<i>P. chrysogenum</i>	$62 \pm 5.0$	$44 \pm 3.5$
Tasmanian	<i>P. italicum</i>	$80 \pm 5.3$	$52 \pm 6.2$

**Table 2** Percentage germination of *A. oligospora* conidia in micro-diffusion tests of allyl alcohol and acrylic acid in presence or absence of silver nitrate solution

Test compound	Silver nitrate solution	
	Present	Absent
Water (Control)	95 ± 2.1	96 ± 1.2
Allyl alcohol (4 p.p.m.)	46 ± 4.6	13 ± 0.8
Acrylic acid (1,000 p.p.m.)	77 ± 3.4	7 ± 2.0

should not be considered as evidence lessening the possible ecological significance of acrylic acid. The microdiffusion system used does not give a direct measure of the potency of the inhibitors but reflects the balance between the rate of diffusion and subsequent accumulation of the inhibitor in the agar films and the rapidity with which spores are germinating. It is, therefore, the relatively high speed with which the conidia of *A. oligospora* germinate and presumably, the low diffusion rate of vapours of acrylic acid, together with a moderate activity, that result in an underestimation of its effectiveness as a fungistatic agent. Thus, when agar films were immersed for a few minutes in acrylic acid solutions and used for germination tests, the effective dose for 50% inhibition was reduced to only 40 p.p.m.

Presence of nutrients counteract the soil fungistatic activity. Similarly, the inhibitory effect of both allyl alcohol and acrylic acid increases progressively with the decrease of the nutrient status of the spores. Note that both the above compounds, within a certain concentration range, depending on the fungus species and the level of nutrients, do not impair the viability of the spores for a considerable period of time (C.B., unpublished).

Brian *et al.*<sup>15,16</sup> have studied the fungistatic activity of a number of ethylene-like compounds and indicated that fungistatic activity is related to the tendency of the substituent groups to withdraw electrons from the double bond<sup>14,15</sup>. This proposition readily explains the contrast in activity between ethylene and allyl alcohol and acrylic acid. The activity of the latter is probably restrained because of the low permeation of cells by organic acids.

My results are in accord with Smith's observation that ethylene causes soil fungistasis<sup>9</sup>, except that the main inhibitor of spore germination is not ethylene but the ethylene-induced and tentatively identified allyl alcohol and perhaps other products. Smith's concept of the oxygen-ethylene cycle<sup>9</sup> may, therefore, represent only part of a more complex system awaiting elaboration.

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## Rhythmic oscillations of phytochrome and its pelletability in *Cucurbita pepo* L.

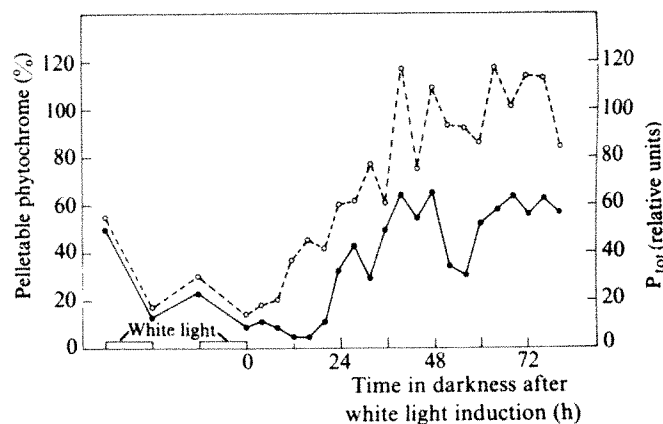
MANY reactions of plants (such as flower initiation, growth and chlorophyll synthesis) show a rhythmically changing sensitivity to light<sup>1</sup>. According to Bünning's hypothesis, the photoperiodic reactions are determined by the interaction between an endogenous rhythm and phytochrome: both components are essential for a photoperiodic response. The endogenous rhythm determines the changing sensitivity of the plant to  $P_{fr}$ , the physiologically active form of phytochrome. These characteristic changes in the sensitivity of the system towards light are probably not attributable to different  $P_r$  molecules. On the basis of the theory that the binding of  $P_r$  to a membrane is the primary reaction of phytochrome, it has been suggested<sup>2</sup> that such sensitivity changes could be achieved by varying the interaction of phytochrome molecules with their receptor sites. We have investigated the association of phytochrome with pelletable structures<sup>3,4</sup> and found that after a 12-h white light induction the total amount of phytochrome and its pelletability oscillate in the dark with frequency periods of 24, 12 and 6 h.

For routine preparations of particulate fractions, pre-chilled tissue—cotyledons or hooks of Squash (*Cucurbita pepo* L., cv. Black Beauty)—were chopped with a razor blade and homogenised with a mortar and pestle in extraction medium. In all experiments the medium-tissue ratio was 3:1(v/w). The medium contained 0.25 M sucrose, 0.1 mM  $MgCl_2$ , 14 mM 2-mercaptoethanol, 25 mM N-morpholino-3-propanesulphonic acid and 3 mM EDTA, pH 8.0. The pH in the final homogenate was about 7.0. The homogenate was squeezed through nylon cloth and recentrifuged at 500g for 10 min after adjusting the pH to 8.0. The supernatant was recentrifuged at 20,000g for 10 min. This supernatant was adjusted to 10 mM  $MgCl_2$ , pH 6.8, irradiated for 5 min with red light and then recentrifuged at 50,000g for 30 min. The pellet was resuspended in the extraction medium and the phytochrome content ( $P_{tot}$ ) was measured in the 50,000g supernatant and 50,000g pellet.

$$\% \text{ Pelletable phytochrome} = \frac{P_{tot} \text{ in } 50,000g \text{ pellet}}{P_{tot} \text{ in } 50,000g \text{ supernatant} + P_{tot} \text{ in } 50,000g \text{ pellet}}$$

$P_{tot}$  was measured at 0 °C with a modified Ratiospect R-2 (ref. 5) using  $CaCO_3$  as scattering agent<sup>6</sup>. All irradiations were carried out at 25 °C. Red light was obtained using a standard red light source<sup>7</sup> (675 W m<sup>-2</sup>). The irradiation with white light was carried out in a phytochamber with xenon arc lamps (7,000 lx).

**Fig. 1** Time course of  $P_{tot}$  (○) and pelletable phytochrome (●) from squash cotyledons in darkness after induction by white light. The first white light exposure began 4 d after sowing.





In the first set of experiments squash seedlings were grown on Vermiculite for 4 d in the dark at 25 °C. After this germination period seedlings received 12 h white light, were returned to the dark for 12 h and then received a further 12 h white light.  $P_{tot}$  and the pelletability were measured in the following dark period every 4 h (Fig. 1).  $P_{tot}$  and pelletability were decreased by the white light treatment. In the dark these variables increased, as expected from earlier observation in which plants were irradiated with red or far-red light<sup>8-11</sup>. In the experimental conditions phytochrome does not seem to be synthesised by a zero-order reaction<sup>8-10,12</sup> but  $P_{tot}$  and pelletability began to oscillate in anti-phase. After about 36 h in the dark they oscillated in phase. The main oscillation period (observed after using the 'moving means' statistical technique) was roughly 24 h.

To investigate the oscillations of phytochrome pelletability in more detail we used the following light regime. Seedlings were grown in the dark for 4 d at 25 °C and exposed to white light for 12 h. To allow sufficient recovery of phytochrome, the first measurement was made after 2 d darkness. In the following dark period phytochrome pelletability was measured every hour for 2 d. The original data of a representative experiment are shown in Fig. 2a; using the three-point moving mean technique

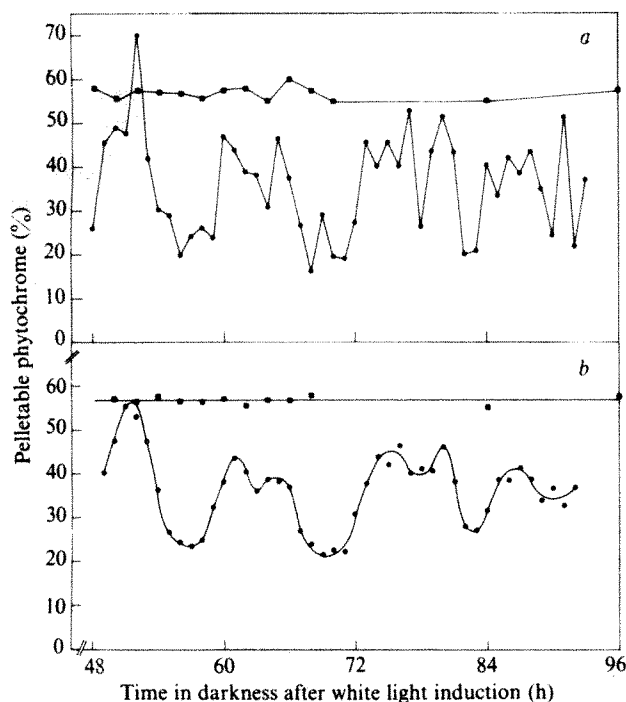


Fig. 2 Time course of pelletable phytochrome from squash cotyledons in darkness after induction by white light (■) or in continuous darkness (●). Seedlings were given only one white light exposure of 12 h.

we obtained the data shown in Fig. 2b. Pelletability oscillated between 20 and 60% and the main period observed was about 12 h. The splitting of the maxima (Fig. 2b) may indicate a second period of about 6 h. The original data indicate the possibility of further oscillations of higher frequencies.

The rhythmic oscillations in  $P_{tot}$  and pelletability were almost certainly attributable to the white light induction period. Without this, pelletability remained constant at about 56%, as shown for the dark control in Fig. 2. Similar rhythmic oscillations could be observed by measuring the pH of the crude homogenate before readjusting it to pH 8.0 (Fig. 3). Again a main period of 12 h and a second period of 6 h could be observed. These oscillations of the pH can be regarded as a marker of the endogenous rhythm of the cell. The oscillation of phytochrome pelletability in nearly the same way as pH oscillations indicates that the rhythm of phytochrome pelletability *in vitro* is not a pure artefact of the experimental method<sup>13</sup>.

These experiments demonstrate a rhythmic change in the ability of pelletable structures to bind phytochrome to 'receptor

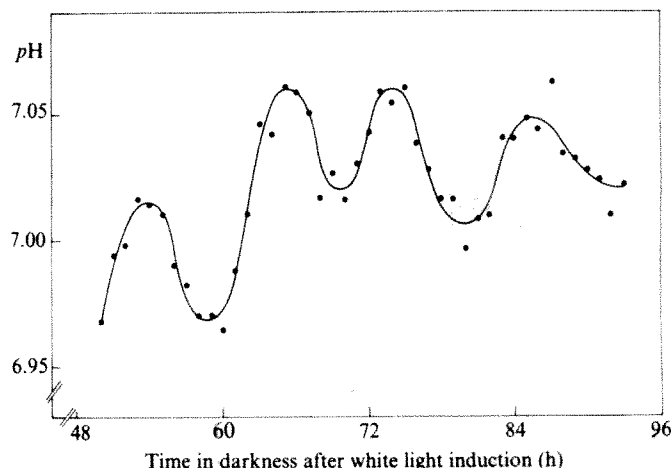


Fig. 3 Time course of changes in pH of crude homogenate from squash seedlings in darkness after induction by white light. Data were calculated using the three-point moving mean technique.

sites'. We cannot decide whether the amount of the pelletable structures or their affinity for phytochrome<sup>4</sup> changes rhythmically.

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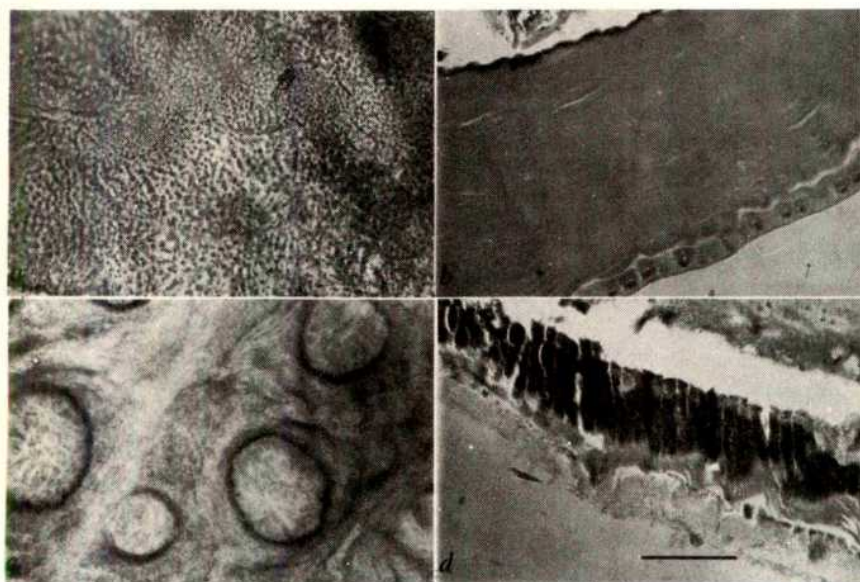
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## Hormonal control of insect epidermal cell commitment *in vitro*

DEPENDING on the hormonal milieu, the insect epidermal cell can express one of several differentiated states manifest by the type of cuticle it secretes. In the Lepidoptera, as long as the juvenile hormone (JH) titre is high, the epidermis secretes larval cuticle periodically in response to ecdysone. When the JH titre declines at the end of larval life<sup>1</sup>, ecdysone causes the secretion of pupal cuticle. In the tobacco hornworm, *Manduca sexta*, two distinct releases of ecdysone are necessary for this metamorphosis<sup>2,3</sup>. The first triggers the cessation of feeding and causes the epidermal cells to switch from a commitment to make larval cuticle to one for pupal cuticle<sup>4</sup>. After this time JH can no longer prevent pupal differentiation. The second ecdysone release begins 2 d later and causes pupal cuticle synthesis. Consequently, during the time of the first release it is possible to study the events which occur to alter the cellular commitment unhindered by those events involved



**Fig. 1** *a*, Surface view of typical larval cuticle produced by implanted epidermis from a feeding larva (< 7 g), or from an *in vitro* culture without hormones or with both ecdysone and juvenile hormone. *b*, Cross section of a typical larval cyst. *c*, Surface view of tanned pupal cuticle showing typical pockmarks formed by an epidermal cyst after exposure to ecdysone *in vitro* or *in vivo*. *d*, Cross section of a typical pupal cyst. Scale equals 100  $\mu$ m.

in the expression of this commitment. I report the hormonal manipulation of this cellular commitment in an *in vitro* system.

The dorsal and lateral integument (epidermis and cuticle) from the third to sixth abdominal segments of a 6.2–7.0 g final instar *Manduca* larva was dissected free of fat body and most of the muscle, cut into pieces (approximately 3×5 mm), and explanted to 0.7 ml of Grace's medium (Gibco) in disposable plastic culture wells (Linbro)<sup>5</sup>.  $\beta$ -Ecdysone (Rohto Pharmaceutical) in 10% isopropanol was either premixed with the Grace's medium or added at an appropriate time. C18JH (>95% the natural *trans, trans, cis* isomer; Eco-Control) was dissolved in Grace's medium by sonication<sup>6</sup> in a siliconised (Siliclad) vessel. For the JH experiments the tissue was incubated in the JH-containing media for 3–4 h before  $\beta$ -ecdysone was added. The cultures were incubated in a 95% O<sub>2</sub>–5% CO<sub>2</sub> high humidity atmosphere on a slow rotary shaker (12–16 rotations min<sup>-1</sup>) at room temperature (23–25 °C) for 21–24 h.

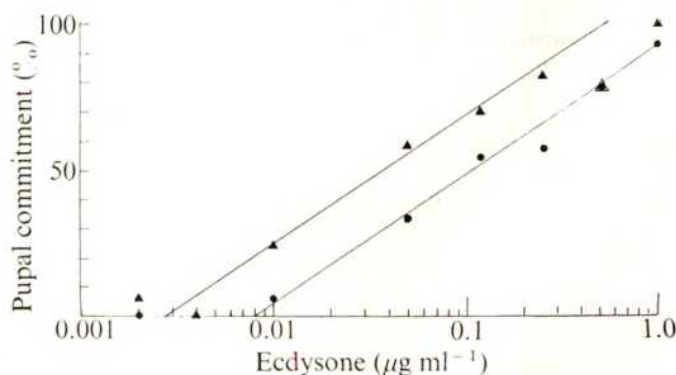
The state of commitment of cultured epidermis was then tested by implantation of the pieces into fourth instar larvae before the initiation of the moult to the fifth stage<sup>7</sup>. One or two days after the host had subsequently ecdysed, the implants were recovered. During *in vivo* culture the implanted epidermis grew around its cuticle to form a cyst<sup>8</sup>. During the moult of the host, the epidermis responded to the host's hormonal milieu by forming a new cuticle on the inside of the cyst. The type of cuticle formed reflected the commitment of the epidermal cells at the time of implantation. When epidermis from fourth instar or feeding fifth instar (up to 7 g) larvae was implanted, the cyst formed larval cuticle which was translucent, rubbery and had numerous papillae on its outer surface when viewed at ×400 magnification (Fig. 1*a*). Furthermore, the epidermis was always deeply pigmented (white or blue) as was typical of larval epidermis. Histological sections showed a thick untanned cuticle (Fig. 1*b*) which was identical to cuticle removed from a normal fifth stage larva. In contrast, when epidermis was taken from wandering fifth instar larvae, the cuticle formed was always pupal in spite of the high concentrations of JH in the host<sup>9</sup>. This pupal cuticle was usually hard, shiny, and either tan or white and often had the typical pockmarks of the pupal abdomen (Fig. 1*c*). Figure 1*d* shows this tanning in the cross section of the cyst. Also, epidermis which has formed pupal cuticle no longer contains pigment granules as is typical of pupal epidermis.

Since the cultured epidermis was taken from larvae weighing less than 7 g, 92% ( $n=174$ ) of control pieces

incubated without hormones formed cysts with >90% larval cuticle. When  $\beta$ -ecdysone ( $1 \mu\text{g ml}^{-1}$ ) was added to the epidermis *in vitro*, 92% ( $n=81$ ) of the implants formed pupal cuticle. As the dose of  $\beta$ -ecdysone was decreased, the percentage of cysts forming more than 90% pupal cuticle declined, as shown by the circles in Fig. 2. Many of the cysts from the intermediate doses showed both larval and pupal cuticle; sometimes only tiny spots of pupal cuticle (the size of one to two epidermal cells) were scattered in otherwise normal larval cuticle. Thus, the switchover from larval to pupal commitment seems to be on a cell-by-cell basis as had already been seen in *in vivo* experiments with JH applications<sup>1,10</sup>. Figure 2 shows that the half-maximal dose of  $\beta$ -ecdysone necessary to commit more than 90% of the cells to pupal differentiation is about  $0.1 \mu\text{g ml}^{-1}$  ( $2 \times 10^{-7}$  M). A dose three times smaller ( $8 \times 10^{-8}$  M) is sufficient to alter the commitment in 50% of the cells, as indicated by the triangles in Fig. 2. These amounts of ecdysone are within physiological range; the dose of  $1 \mu\text{g ml}^{-1}$  necessary to effect complete switchover in commitment is the maximum amount detected in the animal at the time of the first release of ecdysone<sup>3</sup>. Also, these data agree well with the concentration of ecdysone necessary to cause evagination in *Drosophila* imaginal disks<sup>11,12</sup>, development of lepidopteran wing disks<sup>13,14</sup>, puffing in *Drosophila* chromosomes<sup>15</sup>, and moulting of *Manduca* proleg crochet epidermis<sup>16</sup>.

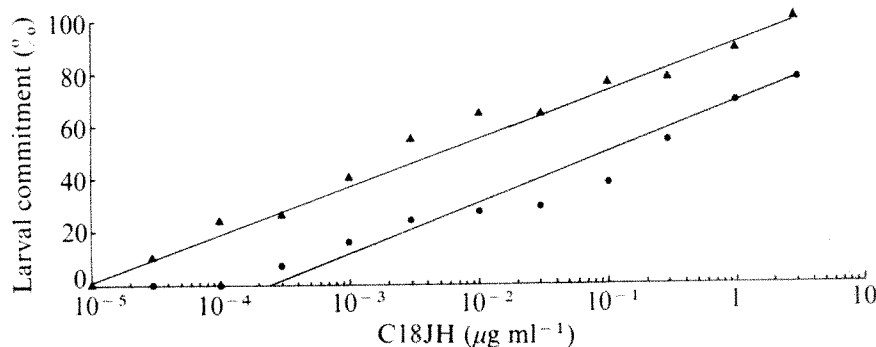
The effectiveness of JH in the prevention of this

**Fig. 2** Changeover in commitment of larval epidermis as a function of the dose of  $\beta$ -ecdysone for 18–24 h *in vitro*. ●, Percentage of cysts showing > 90% pupal cuticle. ▲, Percentage of cysts showing > 50% pupal cuticle. (15–30 cysts per point, except nine at  $0.004 \mu\text{g ml}^{-1}$ .)





**Fig. 3** Inhibition of the changeover in commitment of larval epidermis in response to  $\beta$ -ecdysone ( $1 \mu\text{g ml}^{-1}$ ) as a function of the dose of C18JH *in vitro*. ●, Percentage of cysts showing > 90% larval cuticle. ▲, Percentage of cysts showing > 50% larval cuticle. (15–25 cysts per point, except eight at  $0.1 \mu\text{g ml}^{-1}$ .)



ecdysone-induced change in commitment *in vitro* is shown in Fig. 3. The amount of C18JH necessary to prevent pupal commitment of the epidermis in response to a concentration of  $\beta$ -ecdysone of ( $1 \mu\text{g ml}^{-1}$ ) was  $0.1 \mu\text{g ml}^{-1}$  ( $3 \times 10^{-7}$  M). If the cysts showing >50% larval cuticle are considered, then the 50% dose is lowered to  $2 \times 10^{-8}$  M. The concentration of JH in C18JH equivalents in fourth instar *Manduca* haemolymph before the initiation of the larval moult ranges from  $5 \times 10^{-7}$  M to  $2 \times 10^{-8}$  M (ref. 9). Thus, the concentrations of JH necessary *in vitro* are similar to those found in the intact animal. Also, they are two to three orders of magnitude less than those reported to be essential for inhibition of evagination of leg imaginal disks<sup>17</sup> or for inhibition of cuticle deposition in lepidopteran wing disks<sup>18</sup>, even in the presence of the JH-binding protein<sup>19</sup>. In my cultures little or no JH-binding protein was present because of the virtual absence of fat body which produces it<sup>20</sup>. Apparently, in *Manduca* this protein is not essential for the cellular action of JH as suggested by Sanburg *et al.*<sup>19</sup>. Yet it is possible that the discrepancy found between haemolymph concentration and *in vitro* concentrations could be further reduced by addition of the protein.

This simple *in vitro* system now provides an excellent means for study of the cellular events necessary for the metamorphosis of the insect epidermal cell. When exposed to a physiological dose of ecdysone for 22 h, the cells become committed to pupal differentiation unless a physiological concentration of JH is present. Studies are in progress to elucidate the nature of the cellular events which occur in response to ecdysone. Autoradiographic studies of DNA synthesis in the epidermis during late larval life up to the larval-pupal transformation<sup>5</sup> showed no detectable DNA synthesis in this dorsal abdominal epidermis until 2 d after the switchover of commitment *in vivo* (on day 4). Instead massive DNA synthesis occurs in response to the second surge of ecdysone<sup>2,3</sup> the day before cuticle synthesis begins. Thus, the irreversible transformation from larval to pupal determination in the epidermal cells occurs well before DNA synthesis, and presumably reflects some change in regulatory elements in the cell.

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## Suckling mouse cataract agent is a helical wall-free prokaryote (spiroplasma) pathogenic for vertebrates

THE suckling mouse cataract agent (SMCA) is a filterable organism originally isolated from a pooled extract of rabbit ticks (*Haemaphysalis leporis-palustris*) collected near Atlanta, Georgia in April 1961 (ref. 1). The agent grows to high titre in the eyes and brains when inoculated intracerebrally into newborn mice in which it induces cataract, uveitis and chronic brain infection<sup>1–3</sup>. SMCA has also been grown to high titre in embryonated hen's eggs, in which it produces a lethal infection in 4–9 d (ref. 1). The agent has not been grown in tissue culture or in artificial media, but growth in a rabbit lens organ culture has been reported<sup>4</sup>. Stained SMCA-infected tissues failed to show the presence of rickettsiae and, as a result of such negative inferences and other characteristics of the agent<sup>3–5</sup>, it was considered to be a candidate slow virus<sup>6</sup>. Within the past few years however, ultrastructural and biological studies showed that a wall-free prokaryote was consistently associated with the acute and chronic disease in mice and with lethality in chick embryos<sup>7,8</sup>. Although the agent resembled mycoplasmas morphologically, it differed from them in its apparent non-cultivability on conventional mycoplasma medium. We now report that the organism associated with the suckling mouse cataract syndrome is a prokaryote similar to the spiroplasmas—a group of mycoplasmas known previously only from plants and insects.

The general designation 'spiroplasma' was first given<sup>9</sup> to helical, motile, wall-free prokaryotes observed in corn plants infected with the insect-borne corn stunt disease<sup>10</sup>, even before cultivation of the agents in artificial media<sup>11,12</sup>. Similar morphology was also noted for a cultivable mycoplasma recovered from citrus plants infected with "stubborn disease"<sup>13</sup>. Ultrastructural, serological and biological properties of this organism indicated that it deserved recognition as a new genus and species, *Spiroplasma citri*<sup>13,14</sup>. The absence of cell wall material, and other properties, indicated that this organism belonged to the mycoplasmas (Class Mollicutes). Both of the cultured

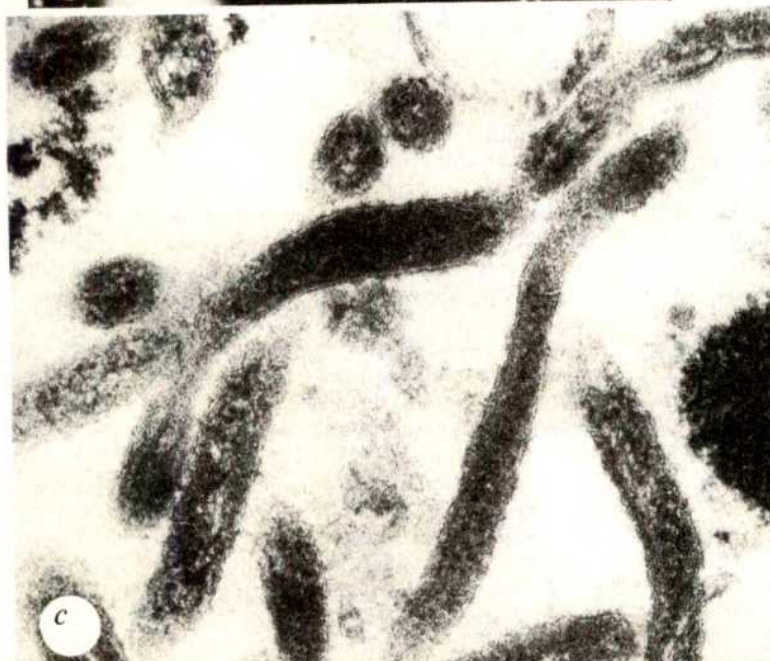
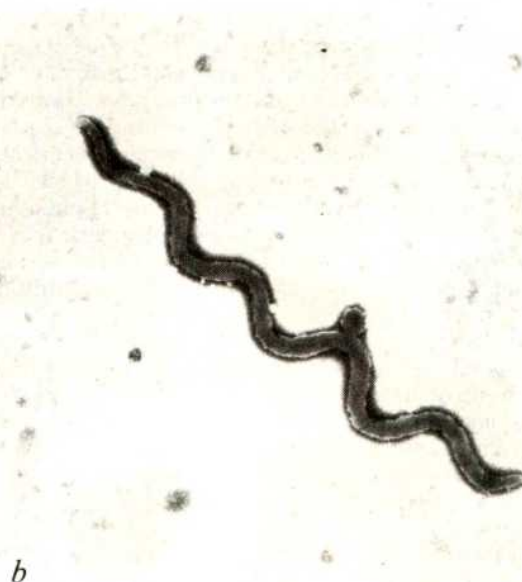
spiroplasmas induce disease in insects<sup>15,16</sup> and plants<sup>11,12,17</sup>. A third possible spiroplasma has been found in natural populations of four closely related species of *Drosophila*<sup>18</sup>. This agent, termed the sex ratio organism (SRO), is inherited maternally and associated with the absence of males in the progeny of infected females<sup>19</sup>. SRO spiroplasmas have not been cultured and are not known to be associated with plants or higher animals.

A pool of frozen ( $-70^{\circ}\text{C}$ ) allantoic fluid from embryonated eggs taken 5–6 d after SMCA infection with about 100 egg lethal doses (ELD<sub>50</sub>) was passaged in 7-d-old chick embryos. Inoculation (0.1 ml undiluted fluid) was made through the yolk sac into 18 embryos; six uninoculated embryos served as controls. Eggs were incubated at  $37^{\circ}\text{C}$  and candled daily to determine embryo viability.

All embryos receiving SMCA died between 6 and 7 d after inoculation. Uninoculated controls, or embryos inoculated with normal allantoic fluids, were still viable at that time. Allantoic and amniotic fluids were collected, from inoculated and control embryos and examined by darkfield microscopy ( $\times 1,250$ ). Motile, helical microorganisms were observed in allantoic and/or amniotic fluids of all embryos inoculated with SMCA, but were never observed in fluids from control eggs. Numbers of

helical organisms varied from 2 to 5 per microscope field to more than 50–60 per field (Fig. 1a). Continued yolk sac passage of infected allantoic fluids, some containing as few as one helical organism per 10 fields, always yielded fluids containing the motile, helical structures after 3–4 d incubation or when the embryo died. At least 10% of embryonated eggs were used as uninoculated controls in each passage but helical organisms were never seen in fluids of these embryos. The agent was subsequently passaged at least five times each in embryonated eggs from special flocks free of detectable pathogens and mycoplasmas (SPAFAS, Inc., Norwich, Connecticut) or from regular commercial flocks (Truslow Farms, Chestertown, Maryland). No differences in the occurrence or yield of motile, helical organisms were apparent in the two egg sources. Egg passages with selected fresh allantoic fluid, containing numerous helical organisms, reduced the mean day of embryo death from 7.0 to approximately 4.0. Allantoic or amniotic fluids collected 3 d after inoculation, and before most embryos succumbed, were relatively clear and usually contained large number of helices.

We also examined a second, tick-derived, mouse brain-passage isolate (GT-48), which is antigenically indistinguishable from SMCA but causes fatal encephalitis when



**Fig. 1** Appearance of SMCA organisms in chorio-allantoic (CA) fluid of embryonated hen's egg 4 d after inoculation through yolk sac. *a*, Photograph of darkfield preparation of CA fluid showing numerous helical filaments. ( $\times 2,800$ ). *b*, Electron micrograph of negatively stained SMCA. CA fluid was fixed overnight in 1% glutaraldehyde (prepared in 0.15 M NaCl and 0.01 M sodium phosphate buffer, pH 7.2). It was centrifuged at 23,000g and the pellet was resuspended in fresh buffered saline. The droplet was picked up on Formvar-coated grid and stained with 1% phosphotungstic acid (pH 7.2). ( $\times 26,250$ ). *c*, Electron micrograph of thin-sectioned pellet of SMCA spiroplasmas. CA fluid was mixed 1:1 with M1 medium<sup>12</sup> and centrifuged at 23,000g. The pellet was resuspended in fresh M1 medium containing final 1% glutaraldehyde, post-fixed in 1%  $\text{OsO}_4$ , dehydrated in graded series of acetone and embedded in Epon. Sections stained with 2% aqueous uranyl acetate and Reynold's lead citrate. ( $\times 124,000$ .)



inoculated intracerebrally into suckling mice and rats<sup>1,7</sup>. Pooled mouse brain extracts were passaged three times by yolk sac inoculation. Again, helical organisms were observed in all inoculated eggs and in none of the control embryos.

Negatively stained organisms and thin sections of fixed organisms were examined by electron microscopy (Fig. 1b and c). The organisms varied in length from 3 to 8  $\mu\text{m}$  and from 0.1 to 0.2  $\mu\text{m}$  in diameter. One end was usually more pointed than the other and no axial structure was visible. Electron microscopy of thin sections of pellets containing SMCA showed a typical trilaminar unit membrane with no outer wall or envelope and no axial filaments or flagella.

The possible serological relationship of SMCA-associated spiroplasmas to plant and insect spiroplasmas was assessed by precipitin ring or deformation tests, techniques previously used to study serological properties of spiroplasmas<sup>18,20</sup>. Preliminary results (Table 1) suggest that the newly discovered spiroplasmas share some antigenic determinants with *S. citri*, corn stunt spiroplasmas, and SRO. These tests also indicate, however, that they are not serologically identical to other described spiroplasmas—findings reminiscent of earlier data on the relationship of *S. citri*, corn stunt organisms and SRO<sup>18,20</sup>.

The helical organisms from chick embryo-derived SMCA and GT-48 are similar in size to other spiroplasmas (3–12  $\mu\text{m}$  long by 0.1–0.2  $\mu\text{m}$  in diameter)<sup>9–13,18</sup>. They also possess typical rapid rotary or 'screw' motion and flexional movements, neither of which yields to translational motion. Finally, we have seen no evidence of cell wall, axial filaments or other structures characteristic of spirochetes or other true bacteria. Thus, the term "spiroplasma"

seems appropriate for their nomenclature. To these commonly accepted criteria we add a property shared by SMCA-associated organisms and spiroplasmas—the presence of infecting viruses. Negatively stained organisms showed helices with attached round bodies or sacs containing numerous virions characteristic of those designated SV-C3 (spiroplasma virus-citri 3)<sup>21</sup>.

Our findings provide substantial evidence that fluids from infected eggs and mouse brain extracts of SMCA and GT-48 which induce ocular or central nervous system manifestations in mice and rats contain large numbers of spiroplasmas. Ultimate proof of the association of these spiroplasmas with the pathological conditions noted in these hosts, and assessment of the relationship of these organisms to established species of *Spiroplasma*, will depend on cultivation of the organisms in an artificial medium and subsequent information on their biological, serological and pathogenic properties. Published data on SMCA and GT-48 infections, however, suggest a direct relationship. Physical properties reported for the infectious entity<sup>1,7,8</sup> correspond closely to expectations for spiroplasmas. Electron microscopic observations on both egg-cultivated SMCA and GT-48 and the involved eye tissues of mice and rats receiving direct challenge with the same egg materials indicated the presence of mycoplasma-like structures<sup>7</sup>. In retrospect, photomicrographs of the agent in both types of tissue showed numerous curved, wall-free organisms similar in structure to spiroplasmas visualised within plant tissue<sup>10,13</sup>, or to suboptimally-fixed spiroplasmas<sup>13</sup>. We have noted similar structures, again retrospectively, in other micrographs<sup>9</sup> of SMCA-infected allantoic fluids.

Evidence that SMCA spiroplasmas came from the tick rests primarily on the isolation of two lines of helical organisms from different tick pools, reisolation of the agent from these pools<sup>1</sup>, and the absence of similar naturally occurring or latent organisms in pools of other tick species collected at the time<sup>1</sup>, or in numerous normal chick embryo fluids examined. The reported isolation<sup>22</sup> of a similar agent (277F) from the same tick species adds further support to a tick origin. Although the 277F agent was not pathogenic for suckling mice (Swiss line), and was at first considered to be a spirochaete, the reported properties now leave little doubt that it is similar to SMCA and other spiroplasmas.

Identification of a spiroplasma associated with, and presumably inciting, pathological conditions in vertebrates, extends the host range and pathogenicity of these organisms. The ability of organisms within this group to reproduce at temperatures from 30 to 37 °C and their multiplication and persistence in the eye, brain and other tissues of some vertebrate hosts, suggests that a search for these new microbial forms in diseases of unknown aetiology may be very fruitful.

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**Table 1** Serological relationships of SMCA spiroplasmas to other spiroplasmas

Rabbit	Antiserum to	Antigen	Ring precipitation (titre)*	Organism deformation (titre)*
480-481	Pre-immune	SC	< 2†	4
	Pre-immune	CSO	< 4	< 2
	Pre-immune	SMCA	< 2	8
	<i>S. citri</i> (SC)	SC	256	5,120
	<i>S. citri</i> (SC)	CSO	64	80-160
	<i>S. citri</i> (SC)	SMCA	32	80
553	Pre-immune	SC	4	4
	Pre-immune	CSO	0	4
	Pre-immune	SMCA	0	16
	Corn stunt organism (CSO)	SC	64	160
	Corn stunt organism (CSO)	CSO	256	2,560
	Corn stunt organism (CSO)	SMCA	64	320
9	Pre-immune	SC	< 4	< 4
	Pre-immune	CSO	< 4	< 4
	Pre-immune	SMCA	< 4	< 2
	SMCA	SC	4	8
	SMCA	CSO	8	4
	SMCA	SMCA	256	640
14	Sex ratio organism (SRO)	SC	4	< 2
	Sex ratio organism (SRO)	CSO	4	< 2
	Sex ratio organism (SRO)	SMCA	64	10

\*Final titre was highest dilution of serum that produced precipitin rings<sup>20</sup> or induced deformation of 50% of helices in at least five microscope fields<sup>18</sup>. Antigens and antisera prepared from or against cultured spiroplasmas for *S. citri* (SC) and corn stunt organisms (CSO), against SMCA allantoic fluid, and SRO *Drosophila* haemolymph.

†Reciprocal of serum dilution.



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## Plaque-forming factor produced by *Mycoplasma pulmonis*

It has been reported that mycoplasma viruses have been isolated from a number of different mycoplasmas—both *Mycoplasma* and *Acholeplasma* species. All the viruses isolated so far, however, only produce plaques on *Acholeplasma laidlawii* lawns and seem unable to infect the mycoplasma from which they were reputedly derived<sup>1</sup>. Some strains of *Mycoplasma pulmonis* are pathogenic for mice and others are not, and because pathogenicity may be attributable to the possession of a virus, we have searched for viruses that may infect *M. pulmonis*.

Initially, five strains of *M. pulmonis* were grown and examined for plaque-forming activity by the "washing method" used for the isolation of acholeplasma viruses<sup>2,3</sup>. All washes were tested on lawns of colonies prepared from each of the mycoplasma strains. In only one case was a

Fig. 1 Growth curve of *M. pulmonis* JB strain and PFF activity  
●, *M. pulmonis*; ○, PFF.

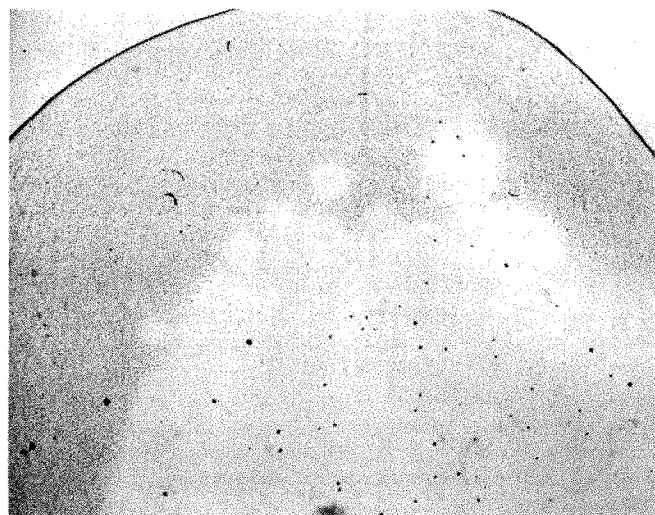
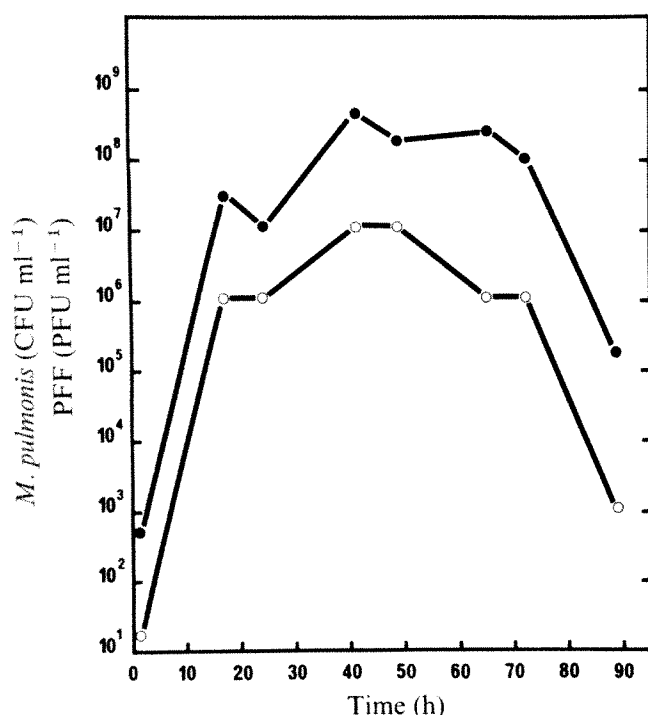


Fig. 2 Plaques produced by *M. pulmonis* JB strain when overlaid with the Peter C strain. Note the mycoplasma colony in the centre of the single plaques.

zone of inhibition or lysis (plaque) seen and this was where the 'JB' strain washing had been dropped on the lawn of the 'Peter C' strain. The plaque, which extended slightly beyond the area of the drop, was faint but distinct. The titre of the plaque-forming factor (PFF) in the JB wash was found to be  $1 \times 10^5$  PFU ml<sup>-1</sup>.

Subsequently, washings from 34 strains of *M. pulmonis* were placed on lawns prepared from 35 strains. Plaques were observed on 96 of the interactions. These plaques were mostly confluent and comprised a central area of the size of the drop, containing mycoplasma colonies (presumably from the washing fluid), and a distinct zone of inhibition around the periphery about 3 mm wide. There were also a few small isolated plaques the largest being only about 1 mm in diameter.

The relationship between mycoplasma and PFF activity was examined by inoculating GS broth<sup>4</sup> with the JB strain and removing samples at intervals during incubation at 37 °C for mycoplasma counting and assessment of PFF activity. The results (Fig. 1) indicate that the PFF titre corresponds closely with the mycoplasma titre, although about 10- to 100-fold lower. Because of the difficulty of counting the small plaques, we devised a more accurate method of examining the relationship between mycoplasmas and PFF activity. Cultures obtained from all of 90 single colonies grown from filtered (450-nm) cultures showed plaque-forming activity, suggesting that as each colony was probably derived from a single mycoplasma cell, each cell therefore possesses plaque-forming activity.

Larger, more distinct plaques were formed when the JB strain was preincubated before being overlaid with the Peter C strain. The inhibition area round the JB colonies increased in size with the length of preincubation until it reached a diameter of 6 mm after 7 d. Where single plaques were visible, one mycoplasma colony, slightly larger than those constituting the lawn, was observed in the centre of each plaque (Fig. 2).

We then investigated the possibility that the area of agar comprising the plaque contained viable mycoplasma organisms. Colonies of JB strain preincubated for 3 d were overlaid with a culture of Peter C strain. After further incubation, plaques 2–3 mm wide developed round the JB colonies. A duplicate which had not been overlaid with Peter C was used to pick samples of agar 0.5–1 mm from the edge of the JB colonies; these samples and the colonies themselves were tested for viable mycoplasmas. Myco-

plasmas were not isolated from the plaques but they were isolated from the colonies.

The plaques may have resulted from inhibition by acid, produced by the JB colonies, so we compared plaque production on normal GS agar and GS agar maintained at pH 8.5 with HEPES buffer. Plaques were produced equally well on both types of agar. It also seemed unlikely that peroxide production by the JB colonies was responsible for plaque formation as plaques developed in an atmosphere of 95% N<sub>2</sub>-5% CO<sub>2</sub>. The failure of catalase (Boehringer), incorporated in agar medium at a final concentration of 10,000 U ml<sup>-1</sup>, to inhibit plaque development confirmed this.

We tried to transmit the PFF, but washings of plaques produced by JB on Peter C lawns produced plaques that were fainter than the original ones and single plaques picked with a straight wire generally failed to produce further plaques.

The size of the PFF was determined by serial filtration of a JB culture under positive pressure of 5-10 pound inch<sup>-2</sup>, through Millipore membranes of decreasing pore size. The mycoplasma titre of the culture before filtration was  $8 \times 10^8$  colony-forming units (CFU) per ml, and  $5 \times 10^7$  CFU ml<sup>-1</sup> and  $1 \times 10^5$  CFU ml<sup>-1</sup> after filtration through 650-nm and 450-nm membranes, respectively. The titre of the PFF was initially about  $2.5 \times 10^7$  PFU ml<sup>-1</sup> and  $5 \times 10^6$  PFU ml<sup>-1</sup> and  $5 \times 10^3$  PFU ml<sup>-1</sup> after filtration through the respective membranes. Neither mycoplasmas nor plaque-forming activity were detected in the 220-, 100- or 50-nm filtrates.

The filtrates were also examined for their ability to inhibit growth of the Peter C strain in a metabolism inhibition test. No inhibition of growth was detected with the 50-nm, 100-nm and 220-nm mycoplasma-free filtrates. The 450-nm and 650-nm filtrates contained too many mycoplasmas for the test to be useful.

A culture of the JB strain, diluted twofold in phosphate-buffered saline (PBS), was held at 56 °C and viable organisms and PFF activity were determined at intervals. As shown in Fig. 3a, the rate of PFF inactivation was about the same as the rate of organism death. A sample (5 ml) of the same JB culture used for heat inactivation was centrifuged and the deposit resuspended in 0.25 ml of PBS. The suspension was diluted 100-fold in PBS, treated with ultraviolet light, and samples taken at intervals for estimation of mycoplasma viability and PFF activity. The results (Fig. 3b) indicate that when the mycoplasmas died, PFF activity was lost. Treatment with Nonidet P-40 (0.4%) for 15 min at 37 °C inactivated the PFF activity. The titre before treatment was  $1 \times 10^6$  PFU ml<sup>-1</sup> and  $< 2.5 \times 10^3$  PFU ml<sup>-1</sup> after treatment. Samples of a culture of strain JB taken at intervals were incubated at 37 °C for 7 h with Mitomycin C (Sigma) or Kanamycin (Kannasyn, Winthrop), at final concentrations of 1 µg ml<sup>-1</sup> and 5 µg ml<sup>-1</sup>, respectively, after which they were examined for mycoplasma viability and PFF activity. Mitomycin C and Kanamycin abolished both mycoplasma viability and PFF activity. To examine the specificity of the plaque-forming activity of the JB strain, tenfold dilutions of a JB culture were placed on GS agar and preincubated at 37 °C for 4 d. Cultures of *A. laidlawii* (strains M1305/68 and BN1), *A. granularum* (Switzer), *A. modicum* (M45-67-1), *M. bovirhinis* (56R and 190), *M. hominis* (3477/44 and 1100/73), *M. gallisepticum* (S6 and A5969) and *M. arthritis* (PG6 and PG27) were poured over the surface of the different agar plates which were reincubated. Confluent lawns developed with all the mycoplasmas, but plaques only developed around the JB colonies overlaid with the S6 strain of *M. gallisepticum*. Centrifuged deposits of a 48-h broth culture of JB strain were examined under the electron microscope by negative staining, or by touch preparations prepared by methods described pre-

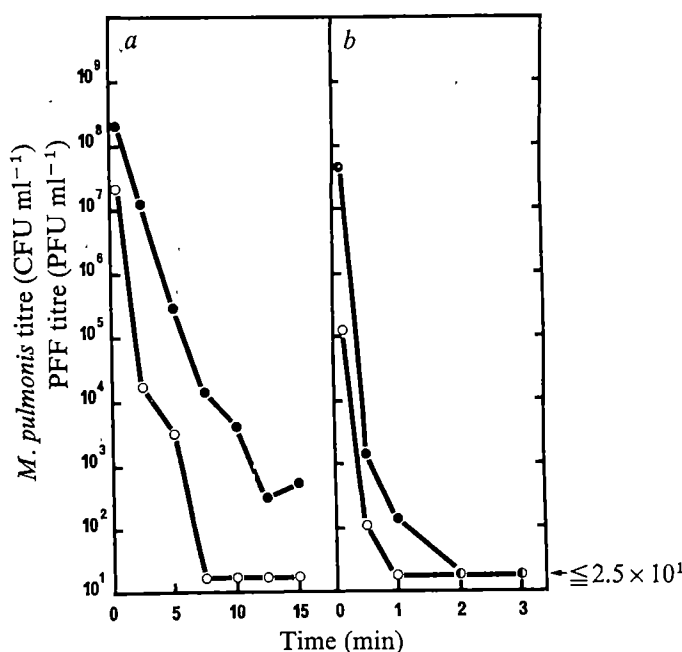


Fig. 3 Inactivation of *M. pulmonis* JB strain and the PFF, by a, heat (56 °C) and b, ultraviolet light treatment. ●, *M. pulmonis*, ○, PFF.

viously<sup>8</sup> Spherical virus-like particles, some about 15 nm in diameter and others about 50 nm in diameter, were frequently observed among the mycoplasma cells. Sections of agar within plaques were also examined but no virus-like particles were seen, so no proof was obtained that the virus-like particles among the mycoplasma cells were associated with plaque-forming activity.

The formation of a plaque on a lawn of the Peter C strain is dependent on the presence of a colony of the JB strain in its centre. In addition, the PFF is inactivated by treatments which also inactivate the JB mycoplasma. The plaque-forming activity also seems to be directly related to the titre of viable mycoplasma organisms as shown by growth and inactivation curves. These observations indicate that the PFF does not develop in the absence of growing JB mycoplasmas. It is intimately associated with the viable JB mycoplasma cell and seems to be inseparable from it. The area of inhibition around a JB colony extends beyond its periphery, however, suggesting that inhibition is due to some diffusible material elaborated by the colony, even though the filtration studies indicate that the apparent size of the PFF is  $> 220$  nm. There is no evidence that such diffusible material is either acid or hydrogen peroxide. The PFF does not have the same characteristics as the mycoplasma inhibitors described by Altucci *et al.*<sup>6</sup> and Furness<sup>7</sup>. Although the PFF seems to have selective activity against certain *M. pulmonis* strains (and *M. gallisepticum*, S6) the inability to propagate plaques serially and to find virus particles within them suggests that plaque formation is not likely to be due to virus production by *M. pulmonis*. The possibility that the PFF is a readily inactivated bacteriocin requires further investigation.

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## Enzymatic basis for DDE-induced eggshell thinning in a sensitive bird

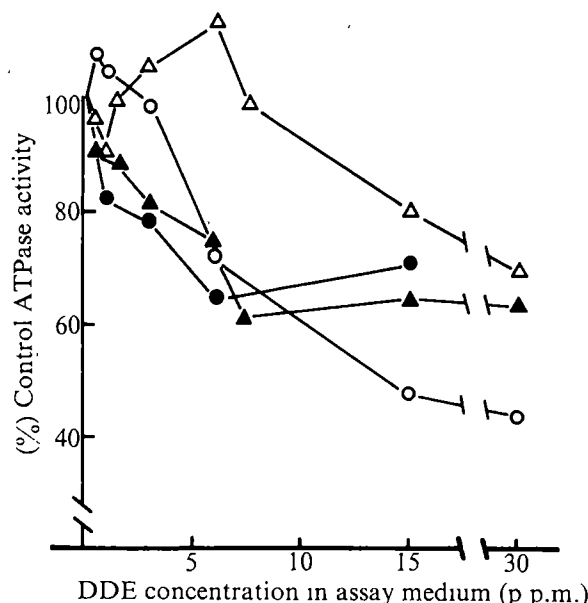
ALTHOUGH the thinning of eggshells as a result of ingestion of DDE [2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene] is well documented in many sensitive avian species, the underlying mechanism remains unclear (see ref. 1 for review). Risebrough *et al.*<sup>2</sup> have proposed that thinning results from inhibitory action close to the site of shell formation since other physiological processes are apparently not affected in brown pelicans laying eggs with up to 95% thinned shells. Our report of no reduction in blood calcium in white Pekin ducks and ring doves exhibiting DDE-induced eggshell thinning<sup>1</sup> supports the proposal that thinning results from a disruption of shell gland (uterus) function rather than a reduced calcium supply to the gland.

The primary function of the avian shell gland is to secrete calcium and carbonate during eggshell formation. Since large quantities of these substances are not stored in the gland, they must be obtained from the blood (calcium) or from metabolic CO<sub>2</sub> (carbonate) and transported to the calcifying egg<sup>4</sup>. As with other tissues which actively transport calcium, for example the intestine<sup>5</sup>, avian shell gland mucosa possesses both calcium-dependent adenosine triphosphatase (Ca-ATPase)<sup>6–8</sup> and calcium binding protein (CaBP)<sup>9</sup>. Mediators of carbonate (or bicarbonate) transport in this tissue include carbonic anhydrase (CA)<sup>4</sup> and, possibly, anion (HCO<sub>3</sub>)-ATPase<sup>10</sup>; other transported-related enzymes include Na,K-ATPase<sup>6</sup> and Mg-ATPase<sup>6</sup>, a part of which we have found to be oligomycin sensitive. The inhibition of one or more of these transport enzymes by DDE could account for the shell thinning observed in sensitive species. Cooke<sup>1</sup> has suggested that there may be several shell thinning mechanisms and that the relative importance of each is species dependent. We have now found that DDE-induced eggshell thinning in ducks is accompanied by decreases in the activities of two shell gland enzymes, Ca-ATPase and CA, the first apparently more important.

Laying white Pekin ducks (which are relatively sensitive to DDE<sup>11</sup>) approximately 9–12 months old were divided into experimental and control flocks and maintained as in ref. 11. White leghorn hens (which are relatively insensitive to DDE and provide a natural control<sup>2</sup>) approximately 12 months old were assigned to control or experimental treatments and maintained in individual cages. Experimental feed was prepared by adding DDE (40 p.p.m.) to mash before making it into pellets. Eggs were collected daily and shell thickness was measured as in ref. 12. To determine shell gland enzyme activities and DDE residues, birds with a calcifying egg in the shell gland were decapitated and the gland was excised. After rinsing in ice-cold, 0.25 M sucrose the mucosa was scraped and the scrapings were homogenised (10–20% weight per volume) in 0.25 M sucrose. Aliquots were frozen rapidly in a dry ice–acetone bath, freeze dried at –20 °C for 24–48 h and stored at –20 °C until use. The ATPase activity in freeze-dried homogenates was determined using a modification of Bonting's method<sup>13</sup> (Fig. 1 legend gives assay conditions); that of CA in freeze-dried homogenates using the method of Maren<sup>14</sup>, that of CaBP in fresh homogenates using the Chelex procedure of Wasserman and Taylor<sup>15</sup>; DDE residues in dried tissue samples were determined using the method of Cade *et al.*<sup>16</sup>. Protein was determined using the

procedure of Lowry *et al.*<sup>17</sup>. As Bonting<sup>13</sup> has noted, and we have confirmed for shell gland mucosa, freeze drying releases maximal ATPase activity from homogenates, and tissue treated in this manner can be stored at –20 °C for at least three months with no loss of enzyme activity. Pritchard *et al.*<sup>6</sup> have obtained a low Ca-ATPase activity in fresh shell gland homogenates from duck, and Pike and Alvarado<sup>8</sup> have observed a loss of Ca-ATPase activity after storage of fresh shell gland microsomes from quail at 0 or –20 °C for 24 h.

Freeze-dried whole homogenates of duck shell gland mucosa had the following ion-activated ATPases: Ca-ATPase, Mg-ATPase, Na,K-ATPase and anion (HCO<sub>3</sub>)-ATPase. As Fig. 1 shows, the addition of DDE to the ATPase assay medium substantially inhibited all ATPase activities. Their *in vitro* sensitivity to low DDE concentrations varied markedly, however, with only Ca-ATPase and Na,K-ATPase inhibited below 3 p.p.m. The fractionation of homogenates before freezing (method of Homan and Schraer<sup>18</sup>) revealed that the most sensitive Ca-ATPase activity was associated with mitochondria (Fig. 2). These *in vitro* dose–response data are perhaps significant in view of the low gland DDE residues (0.75–1.3 p.p.m.) in ducks



**Fig. 1** Inhibition of ion-activated ATPases in duck shell gland mucosa by DDE added *in vitro* to the assay medium. Each point represents the mean of 3–12 ATPase assays on pooled whole homogenates from 2–4 control ducks. Freeze-dried homogenates were reconstituted in 0.25 M sucrose and 0.2 ml aliquots were added to test tubes containing 0.6 ml buffer and salt solutions. ATPase activity expressed as the rate of inorganic phosphate liberation at 37 °C in *V*<sub>max</sub> conditions after correction for non-enzymatic ATP hydrolysis. Five incubation media were used: A,  $5 \times 10^{-3}$  M CaCl<sub>2</sub>,  $3 \times 10^{-3}$  M ATP,  $92 \times 10^{-3}$  M Tris (pH 7.7); B,  $100 \times 10^{-3}$  M NaCl,  $10 \times 10^{-3}$  M KCl,  $3 \times 10^{-3}$  M MgCl<sub>2</sub>,  $3 \times 10^{-3}$  M ATP,  $92 \times 10^{-3}$  M Tris (pH 7.7); C, medium B, except with  $0.1 \times 10^{-3}$  M ouabain; D,  $3 \times 10^{-3}$  M MgCl<sub>2</sub>,  $2 \times 10^{-3}$  M ouabain,  $69 \times 10^{-3}$  M Tris (pH 7.6); E, medium D, except with  $30 \times 10^{-3}$  M Na<sub>2</sub>SO<sub>3</sub>. Ca-ATPase activity was determined in medium A; Mg-ATPase in medium B; Na,K-ATPase as the difference in activity measured in media B and C; anion-ATPase as the difference in activity measured in media D and E. For the anion-ATPase, sulphite was used as it yielded a proportionately greater activity than bicarbonate. The reaction was started by the addition of 0.2 ml of a solution of  $15 \times 10^{-3}$  M ATP and was terminated 10 min later by the addition of 4 ml of ice-cold colour reagent, a strongly acidic solution of 1% ammonium molybdate in 1.15 N H<sub>2</sub>SO<sub>4</sub> in which 40 mg ml<sup>-1</sup> ferrous sulphate had been dissolved before use<sup>13</sup>. After centrifugation, absorbance was measured at 700 nm. DDE was added to the assay medium in *n,n*-dimethylformamide. The final solvent concentration was 0.5–1%, this concentration did not affect enzyme activity. Control rates of ATP hydrolysis were measured in the presence of solvent alone.  $\Delta$ , Mg-ATPase;  $\circ$ , anion-ATPase;  $\blacktriangle$ , Ca-ATPase;  $\bullet$ , Na,K-ATPase.

Table 1 Effect of DDE feeding on duck shell gland ion-activated ATPases and CaBP

	Control	40 p.p.m. DDE	
	4 d	4 d	1-3 months
Shell thickness (mm)	0.483±0.008 (37)*	0.422±0.014 (5)†	0.395±0.008 (17)†
Ca-ATPase	16.5±0.9 (12)	12.6±1.1 (5)†	11.4±0.9 (7)†
Mg-ATPase	25.5±1.7 (12)	21.4±1.5 (5)	21.7±2.2 (7)
Na,K-ATPase	3.9±0.5 (12)	2.3±0.4 (5)‡	4.4±0.7 (7)
HCO <sub>3</sub> -ATPase	4.6±0.4 (11)		4.8±0.6 (4)
CaBP	5.0±1.1 (5)		4.1±0.7 (4)
Carbonic anhydrase	19.5±1.2 (6)		15.8±0.7 (6)‡

\* Values given as mean ± s.e. (*n*), where *n* is the number of eggs or ducks (enzyme data). Data expressed as mm for shell thickness, μmol Pi released per mg protein per h for ATPases, moles Ca bound per mg protein for CaBP and enzyme units per mg protein for CA. ATPase assay conditions as in Fig. 1, except that HCO<sub>3</sub>-ATPase was determined in media D and E with 25 × 10<sup>-3</sup>M NaHCO<sub>3</sub> replacing sulphite in medium E.

† Significantly different from controls, *P* < 0.01.

‡ Significantly different from controls, *P* < 0.05.

laying thin-shelled eggs (Fig. 3). In contrast, Ca binding to gland CaBP was only inhibited by *in vitro* DDE concentrations above 10 p.p.m.: for example, 30% at 27 p.p.m. Finally, Maren *et al.*<sup>19</sup> have reported that there is no inhibition of duck shell gland CA with *in vitro* DDE concentrations as high as 150 p.p.m.

To determine whether duck shell gland enzymes were affected *in vivo*, we fed 40 p.p.m. DDE to laying birds. In agreement with earlier work<sup>11</sup>, this dietary level caused 13±4% (mean±s.e.) shell thinning after 4 d and 18±3% after 1-3 months (Table 1). Thinning was maximal after about 1 week and then remained constant. The activity of Ca-ATPase in whole gland homogenates from these ducks was reduced 24±10% after 4 d and 31±10% after 1-3 months (Table 1) with no tendency to change during the 1-3 months. The only other significant effects (Table 1) were a transient reduction in Na,K-ATPase activity (41±22% after 4 d), which seems to be unrelated to shell thinning because of the return to normal after 1-3 months, and a limited reduction in CA activity (19±8% after 1-3 months), which exceeds that reported previously<sup>6</sup> (10±4% after 2-3 weeks) for ducks fed 40 p.p.m. DDE.

In contrast to the duck, the activity of Ca-ATPase in chicken shell gland was not inhibited by *in vitro* DDE concentrations up to 15 p.p.m. (Fig. 2). Moreover, 2 weeks of feeding 40 p.p.m. DDE did not affect eggshell thickness, any ATPase, or CA (Table 2), even though gland residues were comparable to those found in ducks laying maximally thinned eggshells (Fig. 3). Finally, to determine whether the Ca-ATPase in duck intestine was also unaffected *in vivo*, we fed 40 p.p.m. DDE to male ducklings for 4 weeks. As Table 3 shows, neither Ca-ATPase nor Na,K-ATPase activities were altered, although DDE residues averaged 1.4±0.3 p.p.m. (wet weight whole intestine).

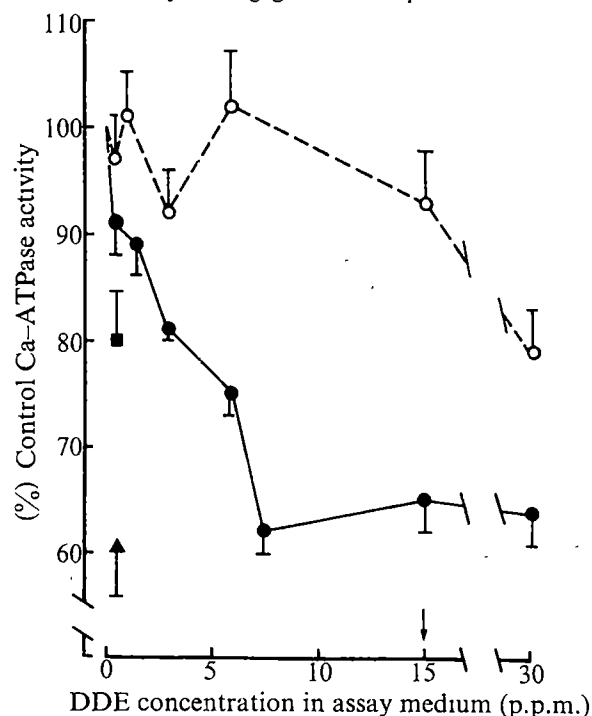
Table 2 Effect of DDE feeding on chicken eggshell thickness and shell gland ATPase activity\*

	Control	40 p.p.m. DDE
Shell thickness (mm)	0.398±0.007 (12)	0.405±0.006 (12)
Ca-ATPase	12.8±0.8 (11)	11.5±0.6 (12)
Mg-ATPase	21.7±1.9 (6)	23.6±1.8 (6)
Na,K-ATPase	3.1±0.6 (6)	2.4±0.8 (6)
HCO <sub>3</sub> -ATPase	3.8±0.4 (6)	4.8±1.4 (6)
Carbonic anhydrase	25.4±2.1 (6)	24.7±3.7 (6)

\* Experimental birds fed 40 p.p.m. DDE for 2 weeks. Values given as mean±s.e. (*n*), where *n* is the number of eggs or chickens. Units and assay conditions as in Table 1. Shell data are for eggs collected on the last day of the experiment; additional data (not shown) indicated no change in control or experimental shell thickness during the experiment.

Whether CA, a soluble enzyme known to be involved in eggshell formation, can be inhibited directly by water-insoluble organochlorines is a matter of controversy<sup>1</sup>. Certainly, duck shell gland CA is insensitive to DDE *in vitro*<sup>19</sup> and the 10-20% decrease associated with maximal shell thinning *in vivo* (ref. 6 and Table 1) may reflect DDE action at cellular sites controlling enzyme synthesis or degradation. Moreover, since CA in many tissues seems to be present in great excess<sup>20</sup>, 10-20% reduction in the activity of duck shell gland suggests a minor role for CA in shell thinning caused by DDE. This mechanism may be

Fig. 2 Inhibition of Ca-ATPase activity in whole homogenates of duck (solid line) and chicken (dashed line) shell gland mucosa by DDE added *in vitro* to the assay medium. Also included are data for crude mitochondrial (▲) and microsomal (■) fractions of duck homogenate. Each point represents the mean of 4-12 assays on pooled homogenates or fractions from 2-4 control ducks or 4 control chickens; variability, when large enough, is given by s.e. bars. Assay conditions as in Fig. 1. Mitochondrial Ca-ATPase activity was reduced to 30% of control by 15 p.p.m. DDE; control activity in both fractions was 3-4 times higher than in whole duck homogenate. Fractions were prepared by differential centrifugation<sup>18</sup> before freezing, and characterised by the presence of generally accepted marker enzymes: oligomycin-sensitive Mg-ATPase for mitochondria and Na,K-ATPase for microsomes. ATPase activity was negligible in the supernatant fraction.





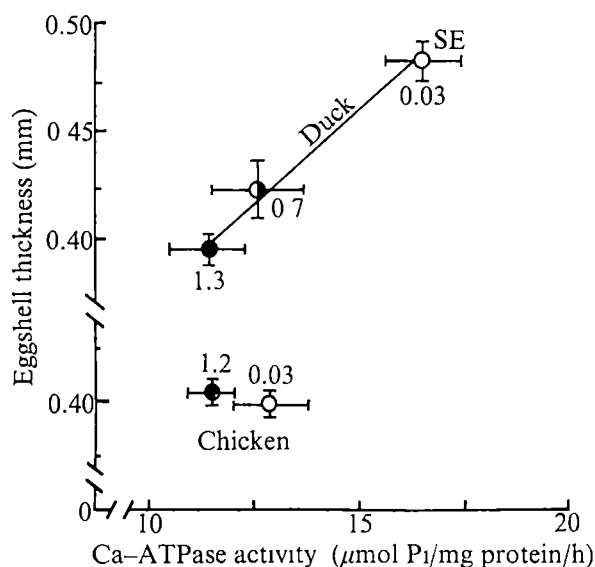


Fig. 3 Difference in *in vivo* sensitivity to both Ca-ATPase inhibition and eggshell thinning following DDE feeding (40 p.p.m.) in ducks (5–12 birds) and chickens (11–12 birds). Residues are means of values obtained from 4–5 birds. Control duck residues taken from ref. 11. ○, Control; ●, 4 d, ● ≥ 2 weeks. Figures show DDE residues (p.p.m.) in shell gland mucosa

more important in other sensitive species, such as the ring dove, in which Peakall<sup>21</sup> has reported a 60% reduction in gland CA activity during maximal DDE-induced shell thinning.

In contrast, all the available evidence points to a major role for Ca-ATPase inhibition by DDE in the shell gland of the duck. Organochlorines often inhibit membrane-bound enzymes such as the ATPases<sup>22</sup>, and the correspondence between *in vitro* and *in vivo* sensitivities to DDE in ducks and chickens (Figs 2, 3) suggests direct Ca-ATPase inhibition with the duck shell gland enzyme being particularly sensitive for unknown reasons. Moreover, unlike CA, transport enzymes such as Na, K-ATPase and Ca-ATPase do not seem to be present in excess, and partial inhibition is generally accompanied by a parallel reduction in ion transport<sup>13,23,24</sup>. Thus, the close correlation between

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## Exposure by phospholipase A of receptors for sheep erythrocytes on human B cells

HUMAN T lymphocytes are known to form rosettes (E rosettes) with certain specific xenogeneic erythrocytes, including sheep erythrocytes (SRBCs). This rosette formation has been widely used as a specific marker for T cells and valuable information has been obtained<sup>1–3</sup>. There is, however, increasing evidence that multiple factors influence the rosette formation; some of these factors can be related to an external effect<sup>4,5</sup> or to the active cell metabolism<sup>6,7</sup>, whereas others are more intimately related to the processes operating during cell maturation<sup>8</sup>. Further, the presence of lymphoid cells carrying E rosette receptors, as well as markers for B cells, has been demonstrated<sup>9</sup>. It has also been demonstrated that neuraminidase treatment induced E rosette formation in a population of lymphocytes bearing B-cell characteristics<sup>10</sup>. Thus, it has not yet been established whether the 'presence of receptor' for E rosette is specific for T cells or not. For this reason, an elucidation of the factors that influence the receptor activity is important if the exact nature of the receptor and the population of cells with it is to be clarified. In the course of investigations on such factors, we have found that treatment of the cells with phospholipase A (PLA) from cobra venom induced the ability to form E rosette in human lymphoblastoid cells that

Table 3 Effect of DDE feeding on duckling intestinal ATPase activity\*

	Control	50 p.p.m. DDE
Ca-ATPase	8.35 ± 0.77 (7)	9.07 ± 0.92 (5)
Na,K-ATPase	3.41 ± 0.23 (9)	3.20 ± 0.92 (9)
Mg-ATPase	11.8 ± 1.2 (9)	15.7 ± 0.7 (9)†

\* Birds fed 40 p.p.m. DDE for 4 weeks. Values given as mean ± s.e. (n), where n is the number of ducklings. Units and assay conditions as in Table 1.

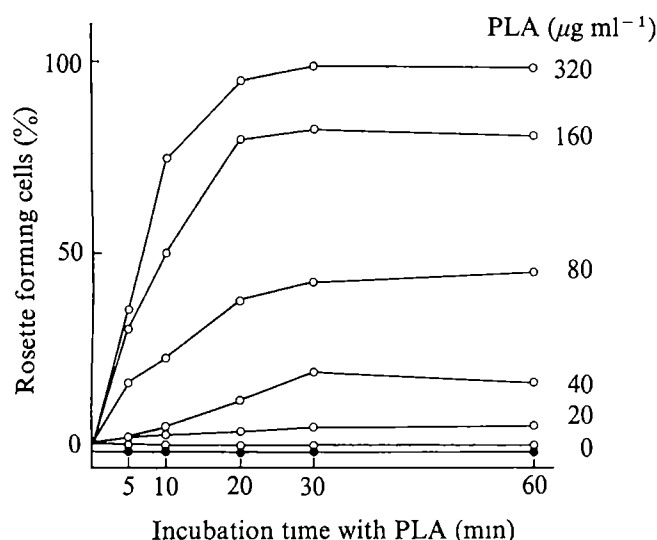
† Significantly different from controls,  $P < 0.05$ .

Ca-ATPase activity and eggshell thickness in ducks (Fig. 3) is consistent with a cause and effect relationship. Finally, although few details of calcium transport in the avian shell gland are known, the mitochondrial accumulation of calcium seems to be involved<sup>25</sup>, and the *in vitro* sensitivity of the Ca-ATPase associated with this organelle (Fig. 2) suggests that DDE, in fact, inhibits a calcium pump in duck shell gland mitochondria.

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are known to be B-cell line. The study also indicated that a population of presumed B cells from the peripheral blood also formed rosettes after such treatment

PLA was purified from lyophilised powder of cobra (*Naja naja*) venom (Sigma) according to the method described by Lankish *et al.*<sup>11</sup> The purified preparation used had an activity unit of 20.8  $\mu\text{mol}$  of released fatty acid per min per mg as assayed using phosphatidylethanolamine (PE) from *Escherichia coli*<sup>12</sup>. The preparations were free from detectable toxic basic proteins and anticomplementary, neuraminidase and protease activities. Human and marmoset lymphoblastoid cell lines were cultivated in RPMI-1640 medium containing 10% foetal calf serum (FCS). Lymphocytes were isolated by Ficoll-Isopaque centrifugation of defibrinated peripheral blood.



**Fig. 1** Rosette formation in Daudi cells treated with PLA. Approximately  $1 \times 10^6$  Daudi cells were incubated at 37 °C in the Tris-HCl buffer, pH 7.2 containing PLA at the indicated concentrations for varying times, washed twice with GVB and then tested for the ability to form rosettes by SRBCs. 0.1 ml of  $1 \times 10^6 \text{ ml}^{-1}$  Daudi cell suspensions were mixed with 0.1 ml of 1.0% SRBCs in the GVB, incubated at room temperature for 5 min and spun at 1,000 r.p.m. for 5 min. After 3 h incubation at 1 °C, the pellet was gently resuspended and the percentages of rosette forming cells (RFCs) were measured (○). Although only the cells with three or more SRBCs were evaluated as RFCs, almost all the RFCs usually had many red cells. All rosette assays were done in duplicate. As a control for 37 °C, Daudi cells treated with 320  $\mu\text{g ml}^{-1}$  of PLA at 4 °C for varying times were tested for the ability to bind SRBCs (●). In this case, even overnight incubation with PLA did not induce rosette formation in the cells.

Daudi cells, as a representative of the human B lymphoblastoid cell lines, were incubated at 37 °C for varying times in the presence of PLA at different concentrations, washed with gelatine Veronal buffer (GVB) and tested for rosette formation by SRBCs. Daudi cells after being treated with PLA, apparently formed rosettes by SRBCs (Fig. 1). The percentages of cells capable of binding SRBCs rapidly increased within 20 min of incubation in the PLA at any concentrations greater than 40  $\mu\text{g ml}^{-1}$  and reached a plateau at 30 min. At that time almost all the cells treated with 320  $\mu\text{g ml}^{-1}$  of PLA could form rosettes. Overnight incubation of Daudi cells in the PLA at 1 °C, however, did not induce any rosette formation. When the SRBCs were incubated at 37 °C for 1 h in the 320  $\mu\text{g ml}^{-1}$  PLA and then tested for their ability to adsorb on the non-treated Daudi cells, no rosette formation was observed. Further, when the mixture of Daudi cells and SRBCs was incubated overnight in the presence of the enzyme at 1 °C and tested for the occurrence of rosettes in the mixtures, no

**Table 1** Rosette formation with SRBC in various tissue culture cells treated with PLA

Cells	Species	Types	% of rosettes§
Daudi*	Human	Lymphoid	95 ± 4
HR 1*	Human	Lymphoid	97 ± 2
Raji*	Human	Lymphoid	99 ± 1
HL 2*	Human	Lymphoid	98 ± 2
MOLT 4†	Human	Lymphoid	96 ± 1
MOLT 4F†	Human	Lymphoid	98 ± 2
HeLa‡	Human	Epithelial	0
HEL‡	Human	Fibroblast	0
B95-8*	Marmoset	Lymphoid	0
LLCMK-2‡	Monkey	Kidney	0
RK-13‡	Rabbit	Kidney	0

\* Before PLA treatment, no definite RFC were observed

† These cell lines have been shown to possess the ability to form rosettes<sup>13</sup>. During the passage, however, the cell lines showed decreased ability to form rosettes. At the present time we cannot detect > 0.1% of RFCs in the culture without pretreatment by PLA, although other characteristics of the cells such as lacking of detectable EBV genome remain unchanged.

‡ These monolayer cells were cultivated in Eagle's MEM containing 10% calf serum. Monolayers were split with trypsin, washed twice with GVB and then treated with PLA. No definite RFCs were observed before PLA treatment.

§ Cells were treated with 320  $\mu\text{g ml}^{-1}$  PLA at 37 °C for 30 min and then tested for rosette formation. Values represent means ± s.d. in 2-3 different experiments

rosettes were found. Heating the enzyme at 100 °C for 15 min at pH 8.5 mostly abolished the rosette-inducing activity of the enzyme, whereas heating at 100 °C for 15 min at pH 6.0 did not change the activity. Thus, it can be concluded that the rosette induction may be produced by direct enzymatic action on the Daudi cells, but may not be produced by the effect on erythrocyte membrane or by the agglutinin-like effect bridging across both the cells because of its nonspecific binding to the cell membranes.

The induction of rosette formation by the PLA was observed only in human lymphoblastoid cell lines. Table 1 shows that all of six human lymphoblastoid cell lines including two MOLT cell lines formed rosettes. In contrast, no rosettes could be formed in the other cell lines regardless of the cell species and types. This suggests that the induction of rosette formation with SRBCs by the PLA may be restricted to the lymphoid cells of human species.

The species specificities of indicator erythrocytes capable of adsorbing on the PLA-treated Daudi cells were tested. The lymphocytes from peripheral blood were also tested for rosettes formation by the same series of erythrocytes (Table 2). Sheep, pig and mouse erythrocytes formed rosettes in 53-70% of the lymphocytes which could be included in the T-cell population. This specificity of the

**Table 2** Comparison with the specificities of erythrocytes capable of adsorbing on the PLA-treated Daudi cells and on the non-treated peripheral blood lymphocytes

Erythrocytes	Daudi*	% Rosettes Lymphocytes†
Sheep	100	70
Pig	100	53
Mouse(dd)	100	68
Human (O)	0	0
Rabbit (albino)	0	0

\* Daudi cells were treated with 640  $\mu\text{g ml}^{-1}$  PLA at 37 °C for 30 min. The washed cells were then tested for the rosette formation by the indicated erythrocytes as described in the footnote of Fig. 1.

† For the rosette assay of lymphocytes,  $1 \times 10^6$ - $2 \times 10^6 \text{ ml}^{-1}$  lymphocyte and 1.0% erythrocyte suspensions were respectively made in the foetal calf serum previously absorbed with the indicated erythrocytes. 0.1 ml of each suspension was mixed, incubated at room temperature for 5 min, and spun at 1,000 r.p.m.<sup>3</sup>. After being incubated at 1 °C for 1 h, the mixtures were gently resuspended and tested for rosette formation.

Table 3 E-rosette induction by various enzymes in Daudi cells

Enzymes	Source	Concentrations ( $\mu\text{g ml}^{-1}$ )	Incubation*		% Rosettes
			min	pH	
Phospholipase A	<i>Naja naja</i>	> 320	30	7.2	> 95
		80	30	7.2	51
	Bee venom†	160–80	30	7.2	3–5
		< 80	30	7.2	0
	Bovine pancreas‡	500–10	30	7.2	0
Neuraminidase§	<i>Vibrio cholerae</i>	100–1 ( $\text{U ml}^{-1}$ )	30	7.0	0
Bromelain	Pineapple	1–0.1 (%)	5	7.2	0
Papain		1–0.1 ( $\text{mg ml}^{-1}$ )	30	7.2	0
Trypsin		0.1–0.01 (%)	30	7.4	0

\* Incubation of cells with enzymes other than trypsin (22 °C) were performed at 37 °C.

† Sigma. Activity unit as assayed by PE: 82.7  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ .

‡ Boehringer Mannheim. 9.2  $\mu\text{mol min}^{-1} \text{mg}^{-1}$ .

§ Worthington.

|| Sigma.

erythrocytes was identical to that of erythrocytes forming rosettes on the PLA-treated Daudi cells.

Several enzymes, including PLA from bee venom and bovine pancreas were also tested for their ability to induce the rosette formation (Table 3). Although bee venom PLA induced rosette formation in a few cells, no other enzymes, including bovine pancreas PLA, could induce a significant rosette formation. It may not be surprising that there was a different effect on the Daudi cells among the three different PLAs. It has been reported that PLAs obtained from these three different sources had different abilities to degrade the phospholipid of human erythrocyte membranes; the erythrocytes were the most sensitive to the *Naja naja* venom PLA, less sensitive to the bee venom PLA and much less sensitive to the bovine pancreas PLA as measured by the degradation of total phospholipid, as well as lecithin, of the cell membranes<sup>14</sup>.

Finally we tested the effect of PLA on E rosette formation in the peripheral blood lymphocytes. The unfractionated and B-enriched lymphocyte preparations were incubated at 37 °C for 30 min in the presence or absence of 320  $\mu\text{g ml}^{-1}$  PLA and, after washing, tested for rosette formation by SRBCs. For the enumeration of B cells the detection of receptors for aggregated IgG was carried out (Table 4). The results show clearly that the values for aggregate binding and rosettes after PLA treatment were apparently above 100%, indicating that a population of B cells, if not all

cells, which possessed the receptor for aggregates acquired the rosette-forming property after PLA treatment.

In conclusion, we have shown that treatment of human lymphoblastoid cells as well as a population of B lymphocytes with *Naja naja* venom PLA resulted in exposure of the receptor(s) for E rosette on the cell. The exposure is probably attributable to the degradation of phospholipids of cell membranes that may cover or competitively inhibit the receptor by a steric hindrance. Earlier studies have demonstrated that the displacement of phospholipids from liver and fat cell membranes by PLA can result in the unmasking of substantial quantities of insulin receptor<sup>16</sup>.

Regardless of the exposure mechanism, our findings and previous studies on the neuraminidase effect on the E rosette formation<sup>10</sup> suggest strongly the possibility that the receptor for E rosette may be present on the human lymphoid cells independently of T or B populations, although, in the latter, it may exist in a state where the activity is masked or inhibited by membrane constituents such as phospholipid and/or sialic acid.

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Table 4 Effect of PLA treatment on E-rosette formation of peripheral blood lymphocytes

Lymphocytes	Receptor for aggregated IgG† %	E-rosette (%)	
		Non-treated	PLA-treated
Unfractionated*			
Case I	28	70	86
Case II	24	73	91
B-enriched†			
Case I	77	15	60
Case II	70	11	65

\* Lymphocyte preparations in RPMI-1640 medium containing 10% foetal calf serum were incubated in the Petri dishes at 37 °C for 1 h to remove monocytes. Thus, lymphocyte preparations used were composed of more than 95% small lymphocytes, less than 5% of monocytes (as judged by peroxidase staining) and almost nil neutrophils.

† The partially purified B-cell population was made by E-rosette formation of T cells and separation of the rosettes by Ficoll–Isopaque gradient as described by Wybran *et al.*<sup>5,15</sup>.

‡ The cells were incubated in the 20  $\text{mg ml}^{-1}$  of heat-aggregated IgG, washed twice with GVB and then stained with FITC-conjugated anti-human IgG ( $\gamma$ ) sera (Dakopatts).

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## Induction of colony formation *in vitro* by human lymphocytes

THE development of a method for colony formation *in vitro* in semi-solid medium by normal macrophages and granulocytes<sup>1-3</sup>, has been a useful aid in elucidating the control of growth and differentiation of these two types of normal white blood cells and the changes in this control that occur in leukaemia and other diseases<sup>4-6</sup>. The formation of these two types of colonies requires the addition of an inducing protein, which we call MGI<sup>5</sup>, that is found in the appropriate serum or condition medium. This inducer does not induce the formation of lymphocyte colonies and it would obviously be of value to obtain colony formation *in vitro* by normal bone marrow-derived (B) and thymus-derived (T) lymphocytes.

Although induction of macrophage and granulocyte colonies does not seem to require addition of a foreign antigen, normal lymphocytes require antigenic stimulation for differentiation in mass culture in liquid medium<sup>7,8</sup>. This suggested that colony formation with normal lymphocytes might be induced in semi-solid medium by providing the appropriate antigenic stimulus. We have now developed a procedure for colony formation *in vitro* with normal human lymphocytes, by stimulating these cells with the lectins phytohaemagglutinin (PHA) or pokeweed mitogen (PWM). PHA has been reported to be mitogenic for T

lymphocytes and PWM mitogenic for both T and B lymphocytes<sup>9</sup>.

Our experiments were carried out with normal human lymphocytes isolated from peripheral blood after centrifugation on a Ficoll-Hypaque gradient<sup>10</sup>. To obtain the appropriate stimulation,  $8 \times 10^6$  lymphocytes were first incubated with PHA or PWM for 1 d in mass culture in liquid medium in Eagle's medium with 25% autologous human plasma and  $10^7$  erythrocytes. After washing the cells,  $5 \times 10^5$  lymphocytes were generally seeded per 35-mm Petri dish for colony formation in soft agar (0.33%) on a harder agar base (0.5%) (ref. 1) in Eagle's medium and 20% autologous human plasma, with the addition of new PHA or PWM to the lower agar layer.

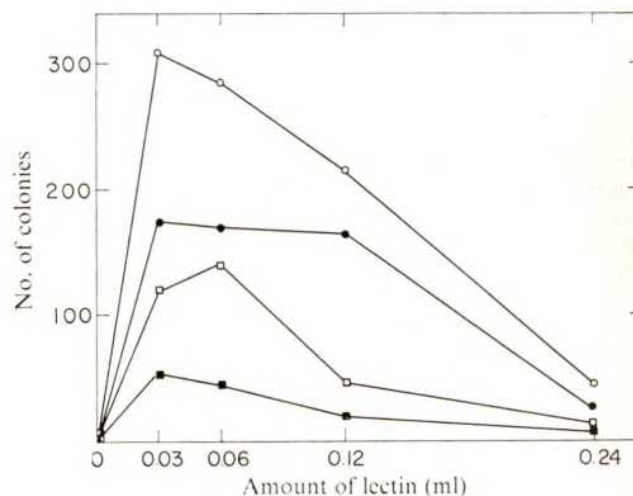


Fig. 1 Photographs of colonies of human lymphocytes in agar induced by PHA. *a*, Type I colonies 4 d after seeding; *b*, type II colony 6 d after seeding. *a*  $\times 125$ ; *b*  $\times 270$ .

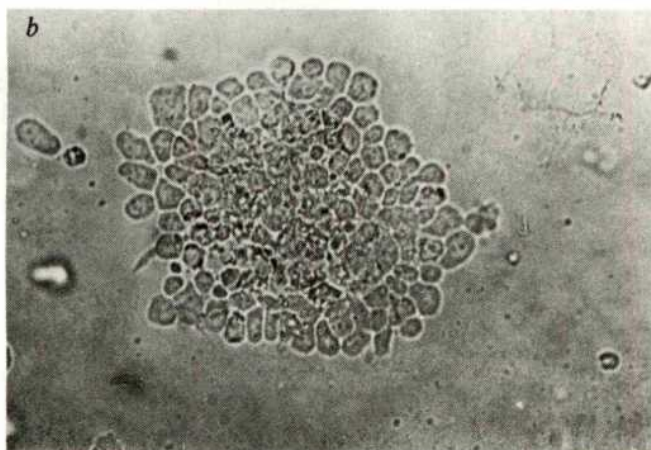
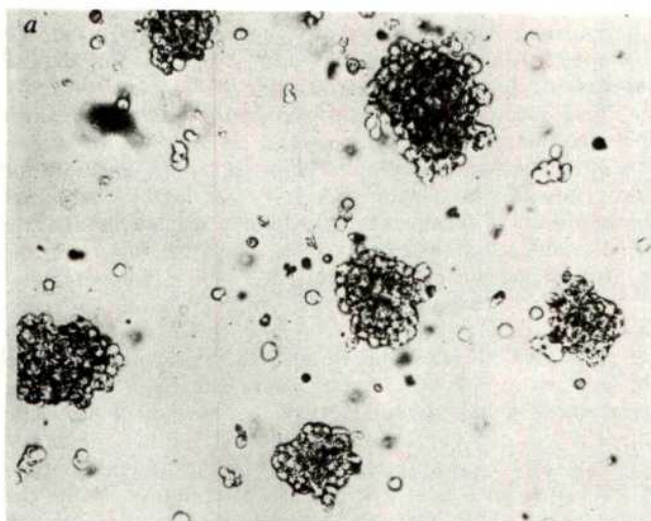


Fig. 2 Number of colonies induced by different concentrations of PHA or PWM. ●, Type I and ○, type II colonies induced by PHA; ■, type I and □, type II colonies induced by PWM. Peripheral blood from healthy adult volunteers was collected with 50 U per ml heparin (pyrogen and preservative free, Evans Medical Ltd). The lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation<sup>10</sup>. Cells from the interphase containing more than 95% lymphocytes were washed 3 times with Eagle's medium with a fourfold concentration of amino acids and vitamins (H-21, Grand Island) and  $8 \times 10^6$  lymphocytes were seeded in  $15 \times 95$  mm plastic tubes with 6 ml Eagle's medium, 2 ml fresh autologous plasma,  $10^7$  autologous erythrocytes and 0.1 ml Bactophytohemagglutinin M (PHA) (Difco) or Pokeweed mitogen (PWM) (Grand Island). After 1 d of incubation at 37 °C in this liquid medium, the cells were washed 3 times with Eagle's medium and  $5 \times 10^5$  lymphocytes were seeded for colony formation in 0.85 ml soft agar (0.33% agar) on a 2.5 ml harder agar base (0.5% agar) per 35-mm Petri dish (No. 1008, Falcon) in Eagle's medium with 20% autologous plasma. Different amounts of PHA or PWM were added to the lower agar layer. Colonies induced by PHA or PWM containing more than 50 cells were counted with an inverted microscope at 5 and 7 d, respectively, after seeding in agar.

Lymphocytes seeded thus gave rise to two types of colonies: the first, which we will call type I, grew inside the upper agar layer (Fig. 1*a*) and the second, type II (Fig. 1*b*) grew on top of the agar. At 5 d after seeding cells in agar with PHA, type I colonies contained up to about 500 cells and type II colonies up to about 200 cells. With PWM, type I and type II colonies contained up to about 200 cells at 7 d. The colonies induced by both lectins had generally degenerated by 10 d and most of the single cells that did not form colonies had degenerated after 3 d. PHA-induced colonies were counted at 5 d and PWM-induced colonies at 7 d. Only colonies containing more than 50 cells were counted. After incubation for 1 d in liquid medium, the cells were agglutinated by both lectins, but these aggregates were dispersed when the cells were washed and there were no such aggregates after seeding in agar. Transfer of both types of colonies to mass culture in liquid medium at 5 and 7 d after seeding with PHA or PWM, respectively, without



adding new lectin, showed that, in liquid medium, the cells from the colonies did not degenerate for at least another 10 d. Cells seeded for colony formation in 1.2% methylcellulose<sup>2</sup> (Dow Chemicals) instead of in soft agar, gave only one type of colony, type I, but the total number of colonies was the same in agar and methylcellulose.

Studies with different concentrations of PHA and PWM have shown (Fig. 2) that the number of colonies formed was related to lectin concentration and that the optimum results were obtained with about 0.03 ml lectin per Petri dish. At this concentration, the total number of colonies with cells from different normal donors was  $491 \pm 98$  for PHA and  $170 \pm 33$  for PWM per  $5 \times 10^5$  cells seeded (Table 1). The number of colonies was related to the number of cells in the range of  $5 \times 10^5$  cells and there were no colonies when the seeding level was below  $10^5$  cells per 35-mm Petri dish (Fig. 3). This suggests that colony formation may require a critical concentration of some factor(s) produced by the seeded cells, in addition to the added lectin.

Mercaptoethanol ( $10^{-4}$ – $10^{-5}$  M), which has been reported to increase the viability of normal lymphocytes<sup>11,12</sup>, did not induce the formation of colonies or enhance the number of colonies induced by PHA or PWM. No colonies were formed when PHA or PWM were omitted either from the preincubation in liquid medium or from the assay for colony formation, indicating that induction of colony formation required the continued presence of the lectin. There were also no colonies, even in the continued presence of the lectin, when autologous human plasma was replaced by foetal calf serum (FCS) in both the preincubation and the colony-forming assay. Substitution of FCS for human plasma only in the assay for colonies, gave at least a 75% decrease in the number of colonies.

Staining of cells from the colonies has shown that both types of colonies induced by PHA or PWM were lymphoblasts, with a similar appearance to the lymphoblasts found after incubation with these lectins in liquid medium. These lymphoblasts did not show phagocytosis of agar. The present procedure did not induce the formation of macrophage, granulocyte<sup>1-3</sup> or erythroid<sup>13</sup> colonies. The formation of lymphoblast colonies by PHA has also been obtained in other experiments with lymphocytes from bone marrow, spleen or peripheral blood<sup>14</sup>.

Cells from the lymphoblast colonies in agar were tested for their ability to form rosettes with sheep erythrocytes (E rosettes) that are formed with human T lymphocytes<sup>15-17</sup> and with sheep erythrocytes coated with antibody (EA

**Table 1** Number of colonies with peripheral blood lymphocytes from five different normal donors

Donor no.	PHA		PWM	
	No. of colonies Type I	Type II	No. of colonies Type I	Type II
1	188	320	55	102
2	131	199	24	90
3	211	423	45	127
4	165	350	66	140
5	155	312	71	129
Mean $\pm$ s.d.	170 $\pm$ 28	321 $\pm$ 72	52 $\pm$ 17	118 $\pm$ 18

The cells were incubated in liquid medium with 0.1 ml lectin and seeded in agar with 0.03 ml lectin. The number of colonies are per  $5 \times 10^5$  cells seeded. In the cultures in liquid medium, there were  $71 \pm 10\%$  blast cells with PHA and  $53 \pm 7\%$  blast cells with PWM, 3 d after seeding.

rosettes) or with antibody and complement (EAC rosettes) that are formed with B lymphocytes<sup>18</sup>. Since cells from isolated colonies were surrounded by considerable amounts of agar even after washing, pooled colonies of both types were incubated overnight in liquid medium, washed and then tested for their ability to form rosettes. Many of the cells from PHA-induced colonies formed aggregates in the rosette assays, but none of the PWM-induced colonies did so. In the assay for E rosettes, there were also some erythrocyte aggregates in the incubations with cells from PHA-induced colonies, but not from PWM-induced colonies. The quantitation of rosette-forming cells was, therefore, more reliable with the cells from PWM-induced colonies. They contained about 60% cells with E and about 25% cells with EA and EAC rosettes. About 10% of the cells were stained for surface immunoglobulin (Ig) by fluorescent anti-human IgG or the Fab fragment of IgG (Miles-Yeda). These results indicate that the PWM-induced colonies formed *in vitro* contained T and B lymphocytes. The PHA-induced colonies contained 60% cells with E rosettes and showed no staining for surface immunoglobulin.

These results, showing *in vitro* colony formation with normal human lymphocytes, should be of value for studies on antigenicity, the immune response and the results of virus infection and lymphocyte diseases. Our finding of optimum colony formation with human plasma should also be of value for studies on the role of plasma factors in the control of growth and differentiation of lymphocytes.

Since these experiments were completed, it has been reported<sup>19</sup> that colonies of lymphoid cells with the B-cell markers, EA rosettes and fluorescent staining for surface Ig but without EAC rosettes, were obtained by adding mercaptoethanol to cultures of mouse haematopoietic organs. We did not obtain colonies by adding mercaptoethanol to isolated normal human peripheral blood lymphocytes, and in our experiments, stimulation with the appropriate lectin produced colonies containing cells with markers for T and B cells.

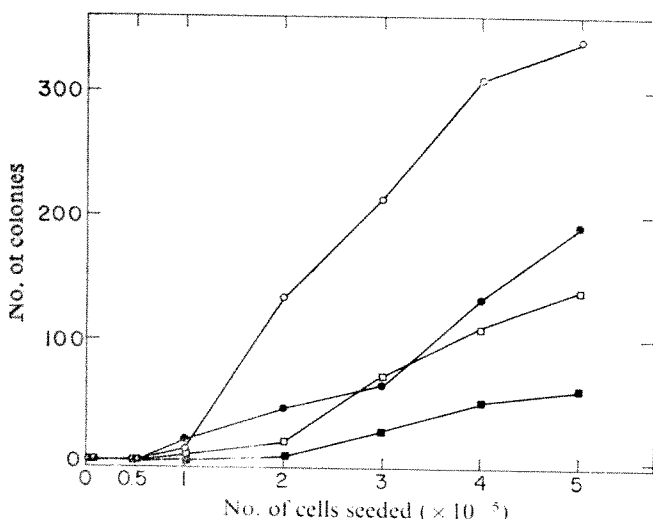
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**Fig. 3** Relationship between number of colonies and number of cells seeded. ●, Type I and ○, type II colonies induced by PHA; ■, type I and □, type II colonies induced by PWM. Cells were incubated for 1 d in liquid medium with 0.1 ml lectin and then seeded for colony formation with 0.03 ml lectin.



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## Colony formation *in vitro* by leukaemic cells in acute myelogenous leukaemia with phytohaemagglutinin as stimulating factor

HUMAN bone marrow contains cells which form leukocyte colonies of 50 or more cells. Each such colony arises from a single cell<sup>1</sup>, belonging predominantly to the myeloid series<sup>2,3</sup>, which is subject to regulation *in vitro* by humoral colony-stimulating factor generated by peripheral leukocytes<sup>4,5</sup>.

Bone marrow cells in untreated acute myelogenous leukaemia (AML) show abnormal growth *in vitro* in the Robinson assay<sup>6</sup>. Characteristically, there is near total failure of colony formation; predominantly clusters are formed containing 20 cells or less<sup>7,8</sup>. Because such poor proliferation *in vitro* might not represent maximum *in vitro* and *in vivo* proliferation of the leukaemic cells, we have studied several modifications of the *in vitro* culture technique. We report here an *in vitro* system in which marrow cells from untreated AML and AML in relapse were stimulated by phytohaemagglutinin (PHA) to form leukaemic cell colonies in soft agar.

Basically, the PHA assay consists of two phases; an initial liquid phase of 15 h at 37 °C and a semi-solid period of 7 d incubation at 37 °C. In the liquid phase  $2 \times 10^6$  marrow cells per ml of medium (Dulbecco's minimal essential medium + 10% foetal calf serum (FCS) + 10% human serum (HS)) were cultured in Pyrex glass tubes to which 0.05 ml PHA (Difco, PHA-M) per ml medium was added. Bone marrow cells were obtained by aspiration from the iliac crest. The technique used for preparing a marrow cell suspension suitable for tissue culture has been described before<sup>9</sup>. After 15 h of incubation, the cells were washed twice using Hanks' balanced salt solution (305 mosM) and resuspended in agar medium (final concentration agar 0.25%, medium Dulbecco's MEM + 10% FCS + 10% HS). After being resuspended in 0.2 ml agar medium,  $1 \times 10^5$  cells were pipetted into 35-mm Falcon Petri dishes containing 1 ml agar medium (final concentration agar 0.5%, medium Dulbecco's MEM + 10% FCS + 10% HS) to which  $1 \times 10^6$  peripheral blood leukocytes from normal individuals were added using the method of Robinson<sup>10</sup>. Simultaneously,  $1 \times 10^5$  cells were plated in Petri dishes containing agar underlayers without leukocytes. After 7 d of incubation in a 7.5% CO<sub>2</sub> gas-controlled humidified incubator at 37 °C, colonies were visible microscopically. Using an inverted

microscope, the colonies were counted. Aggregates containing 50 cells or more were considered colonies.

Using this PHA assay, we tested the growth of marrow cells from both untreated AML patients and AML patients in relapse. In both cases, the blast cell concentration in the bone marrow exceeded 70%. Moreover, in simultaneous experiments, marrow was tested from leukaemic patients in remission and from haematologically normal individuals. Table 1 summarises the results and shows that *in vitro* colony formation of the marrow from 14 untreated AML patients and from four AML patients in relapse did not occur in the conventional Robinson system. In contrast, colonies were observed in the *in vitro* PHA assay. It seems



**Fig. 1** Ultrathin cross section of a cell in a 7-d-old colony in the PHA assay from marrow of a patient with AML relapse ( $\times 6,750$ ). The nuclear pocket (arrow) and the peroxidase-positive granules are clearly visible. The nuclear pocket is consistent with chromosome abnormalities in the leukaemic cell population<sup>11</sup>.

from the results in Table 1, column 5 that the presence of leukocytes was not essential for colony formation by leukaemic marrow. This contradicts the data of Metcalf *et al.* and Moore *et al.*, who observed no dependence of the presence of leukocytes *in vitro* for leukaemic cell proliferation<sup>11,12</sup>. An explanation for the discrepancy between our results and the Australian data might be that PHA stimulates a different cell sub-population of the leukaemic pool.

**Table 1** Mean number of marrow colonies from AML patients and haematologically normal individuals in the Robinson assay and the PHA assay

Bone marrow	No. of patients	Leukocyte feeder*	Leukocyte feeder	Agar†
Untreated AML	14	0 (0)	99 (14–290)	113 (22–440)
Relapse AML	4	0.5 (0–2)	125 (52–160)	90 (0–130)
Remission AML	10	22 (5–44)	16 (8–46)	0.16 (0–1)
Haematologically normal	5	34 (25–44)	29 (22–35)	0 (0)

\*Mean number of CFU-C per  $10^5$  marrow cells plated. Figures in parentheses represent the range of colonies obtained.

†Cells plated on agar underlayers without leukocyte feeder.



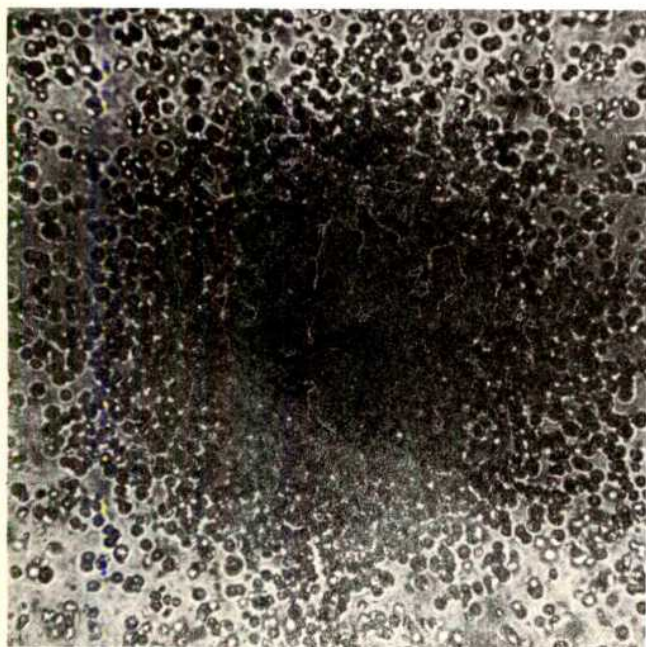
The relationship between the number of cells plated and the number of colonies obtained which was determined in one patient, was linear. This and additional dose-response curves to confirm this observation will be presented later.

Table 1 shows that bone marrow cells in the remission phase of AML form colonies in the Robinson assay as well as in the PHA assay provided that leukocytes are present. The number of colonies scored in the Robinson system is equivalent to that in the PHA assay, indicating no additional effect of PHA on colony formation by marrow cells in remission. Moreover, these data clearly demonstrate the absolute dependence on the leukocyte factor for colony formation by the remission marrow. The same dependence prevails for colony formation of marrow cells from haematologically normal individuals. The similarity of *in vitro* growth between remission marrow and haematologically normal marrow is further evidence for their identity.

Evidence for the leukaemic origin of the bone marrow colonies from untreated AML patients and from AML patients in relapse was obtained by chromosome analysis of cells in the colonies and from morphological studies at the ultrastructural level. These detailed studies will be published soon. Figure 1 shows an electron micrograph of a cell in a colony from the bone marrow of an untreated AML. The nuclear pocket or bleb is apparent on the surface of the nucleus. This morphological alteration is consistently observed in leukaemic cells demonstrating an abnormal chromosome pattern<sup>13</sup>. The karyotype of the leukaemic cell population in this patient was 45,X,-Y,-C,+D,+E,-G, a complex pattern which has been observed in other acute leukaemic patients<sup>14</sup>. Figure 2 shows a colony obtained from the bone marrow of an untreated AML patient. This particular colony consists of approximately 500 cells.

This is a first report of PHA-induced mitogenesis of human leukaemic cells *in vitro*, which is distinct from agglutination of malignant cells by lectins<sup>15,16</sup>. The cultures were checked for cell agglutination immediately after plating. Although in two cases clumps of 2-10 cells were present, in all other cases bone marrow suspensions were distributed in agar as single cells. For this reason further research in technology is needed before an exact assessment can be made of PHA stimulation in every leukaemia.

Fig. 2 Colony cells (7 d old) from an untreated AML in the PHA assay. In this particular colony, 500-700 cells are present ( $\times 45$ ). The leukaemic origin of the colony has been established by electron microscopy.



Aggregation by cellular migration after culture was excluded by counting the number of aggregates in the PHA assay and by comparing that with the number of aggregates in the Robinson system, which was done in eight cases. In all eight cases the total number and size of aggregates in the PHA assay exceeded those in the Robinson assay, which suggests that PHA induced greater cellular proliferation than occurs in the Robinson assay.

Although the mode of action of PHA on the leukaemic cell population is not known, PHA seems to stimulate leukaemic cells to form colonies. But cell types other than leukaemic cells may survive in the PHA *in vitro* cultures. In spite of this, the *in vitro* phenomenon described here demonstrates the growth potential of a leukaemic cell population. It is very important to study whether or not the leukaemic stem cell *in vivo* belongs to the leukaemic subpopulation which is sensitive to PHA *in vitro*. Meanwhile, this *in vitro* phenomenon may be useful in the detection of residual leukaemic cells during the remission phase of AML.

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## Development of colonies *in vitro* of mitogen-stimulated mouse T lymphocytes

TECHNIQUES have been developed for the growth of haemopoietic cells (see refs 1 and 2), human T lymphocytes<sup>3</sup> and mouse B lymphocytes<sup>4</sup> on soft agar. Experiments with mouse lymphocytes suggest that concanavalin A (con A) and phytohaemagglutinin (PHA) stimulate T cells<sup>5,6</sup>, whereas pokeweed induces proliferation of both B and T cells<sup>7</sup>. Based on previously described techniques for obtaining clonal growth of human lymphocyte colonies *in vitro*<sup>3</sup>, a procedure has now been developed for inducing clonal proliferation of mitogen-stimulated mouse lymphocytes using a two-layer soft agar technique.

Cells were obtained by removing inguinal and mesenteric lymph nodes and the spleen, in aseptic conditions, from mice of strains ICR, C3H/HeJ, C57BL/6 and congenitally athymic (nu/nu) mice.

The organs were crushed, and passed through stainless steel mesh into cold phosphate-buffered saline. Cell suspensions in Eagle's medium (EM) were cultured at 37 °C in a water-saturated atmosphere with 5-7.5% CO<sub>2</sub> for 48 h in a concentration of 5 × 10<sup>6</sup> per ml EM containing PHA-M (Difco, 0.0125 ml ml<sup>-1</sup>), or con A (Difco, 30 µg ml<sup>-1</sup>), and 5% fresh normal human pooled serum which had been heat inactivated at 56 °C for 30 min. The



mitogen-stimulated lymphocytes were washed twice in EM and the resuspended cells were seeded in the upper agar layer,  $10^6$  cells to each 30-mm Petri dish. The lower agar layer consisted of 2.5 ml medium containing modified Eagle's medium, 20% heat-inactivated human serum, 0.5% agar and  $0.0125 \text{ ml ml}^{-1}$  PHA or  $30 \mu\text{g ml}^{-1}$  con A.

The upper layer (0.85 ml) consisted of EM, 20% inactivated human serum, 0.32% agar, and the seeded lymphoid cells. This was incubated at  $37^\circ\text{C}$  in a humidified atmosphere with 5–7.5%  $\text{CO}_2$ . As controls, cultures were set up in which the lower agar layer did not contain mitogen, or the lymphoid cells present in the upper layer had not been sensitised with PHA or con A.

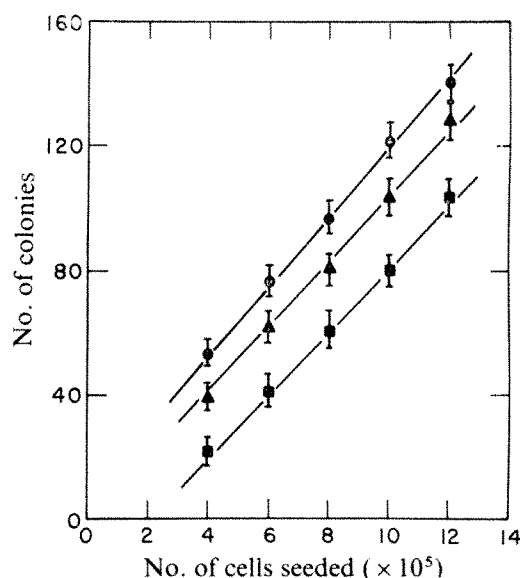
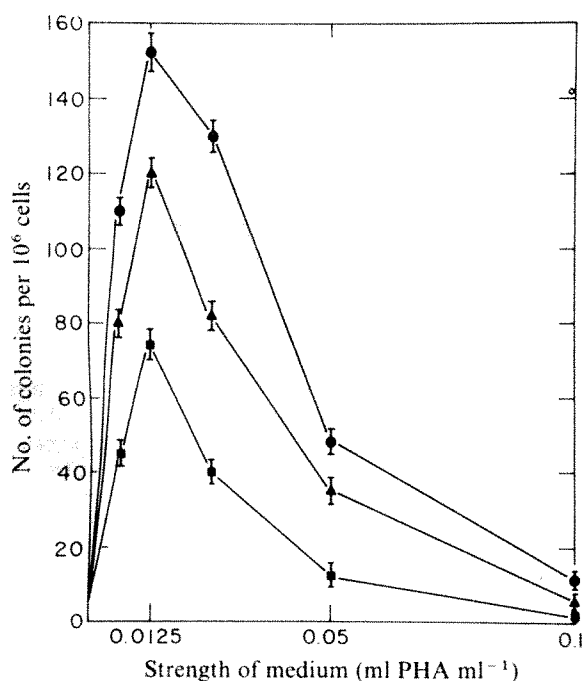
The development of colonies and their morphology were observed under an inverted microscope at  $\times 50$ . The majority of seeded cells underwent lysis during the first and second days of culture, while the surviving cells proliferated and developed into colonies. Through days 3–5 of culture, each colony was made up of at least 50 cells, with most colonies reaching larger size and containing about 1,000 cells. Following a stationary period of 2–3 d the colonies began to degenerate on day 7 or day 8, and on day 11 the colonies showed complete disintegration with lysis of the cells.

Studies of the cell morphology of the colonies which appeared after 3–4 d, showed all cells to be large and pyroninophilic, resembling in their ultrastructure the blast-like cells which are observed on culturing lymphoid cells in a liquid medium in the presence of PHA or con A. A mitotic index of 5–6% was determined in colony cells after 5 d on soft agar cultures.

When spleen cells were seeded in addition to lymphocyte colonies, macrophage and granulocyte colonies developed, at the same time or 1–2 d later. Also, in some experiments a new kind of colony, composed of large macrophages, developed after days 12–14 and increased in size to day 20.

Similar to the ability of lymphocytes to produce a factor stimulating the growth of bone marrow macrophages and granulocytes<sup>4</sup>, PHA- or con A-sensitised murine lymphocytes in soft agar cultures produced a colony-stimulating factor which may have stimulated the macrophage colonies.

**Fig. 1** Effect of varying the concentrations of PHA in the lower agar layer, and of human pooled heat inactivated serum in the agar layers on development of colonies. ●, 20% human pooled serum; ▲, 10% human pooled serum; ■, 5% human pooled serum.



**Fig. 2** A linear relationship between the number of cells seeded and the number of resulting colonies with either PHA ( $0.0125 \text{ ml ml}^{-1}$ ) or con A ( $30 \mu\text{g ml}^{-1}$ ) in the culture were used. For the same mouse strains, the number of colonies was greatest when PHA was used as mitogen. ●, PHA, ICR; ▲, PHA, C3H/HeJ; ■, con A, ICR.

The number of lymphocyte colonies developing in specific experiments, depended on the mitogen used, the organ of origin, and other specific conditions. We obtained our best results with 20% inactivated freshly pooled human serum, while  $0.0125 \text{ ml}$  PHA or  $30 \mu\text{g}$  con A per  $\text{ml}$  medium gave the best cell preservation, and facilitated the development of large numbers of colonies (Fig. 1). An approximately linear relationship was found between concentration of PHA in the agar layer and number of developing colonies for low concentrations; concentrations exceeding  $0.0125 \text{ ml ml}^{-1}$  depressed colony formation (Fig. 1).

Presensitisation with the mitogens, as well as their continuous presence in the soft agar, were essential conditions for colony formation. Incubation periods with PHA in liquid medium exceeding 48 h did not cause any further significant increase in the number of developing colonies.

A linear relationship was observed between number of cells seeded and number of colonies developing, regardless of whether different mouse strains or different mitogens were used. The potential for colony formation of PHA, in comparison, was higher than that of con A in the same conditions of culture (Fig. 2).

In spite of the fact that a great number of lymphoid cells were seeded ( $10^6$  cells per plate), the ability of clonal proliferation was limited to a minority of transformed lymphocytes. No lymphocyte colonies developed when con A- or PHA-sensitised lymph node or spleen cells of congenitally athymic (*nu/nu*) mice were cultured (Table 1).

**Table 1** The largest number of T-cell colonies were obtained culturing inguinal lymph node cells from different mouse strains using PHA as mitogen

Mouse strain	Mitogen	Lymphocytes prepared from lymph nodes	Number of colonies/ $10^6$ cells (mean $\pm$ s.e.)
ICR	PHA	Inguinal	$120 \pm 7.4$
ICR	PHA	Mesenteric	$80 \pm 8.2$
ICR	Con A	Inguinal	$80 \pm 5$
C3H/HeJ	PHA	Inguinal	$110 \pm 8.2$
C57BL/6	PHA	Inguinal	$170 \pm 16$
C57BL/6	PHA	Mesenteric	$62 \pm 6.4$
congenitally athymic ( <i>nu/nu</i> )	PHA or Con A	Inguinal or Mesenteric	0

No colonies were produced by culturing congenitally athymic (*nu/nu*) mice cells known to be markedly deficient in functional T cells.



To identify the nature of the colony-forming cells, indirect immunofluorescent staining of the  $\theta$  isoantigen<sup>9</sup> was carried out on cells pooled from colonies obtained after 4–5 d of culture of C3H/HeJ lymph node cells. Colonies were removed with capillary tubes, washed three times with Ringer solution containing 5% inactivated human serum, incubated with anti- $\theta$ , washed once more with Ringer solution–human serum; bound anti- $\theta$  was detected by using fluorescein isothiocyanate conjugated rabbit anti-mouse immunoglobulin. The  $\theta$ -bearing cells were revealed by appearance of bright ring-like fluorescence. Demonstration of the  $\theta$  isoantigen in cells of colonies, as well as the failure to obtain cultures from congenitally athymic nude mice, known to be deficient in T cells, indicate that con A- or PHA-stimulated cells capable of developing into colonies in the soft agar system belong to the T-cell series.

This technique for growing and developing mouse T-cell colonies should be valuable for the investigation of lymphoid cells. Its particular advantage is to allow the study of a lymphocyte colony population derived from a particular lymphocyte series possessing the ability to develop into colonies.

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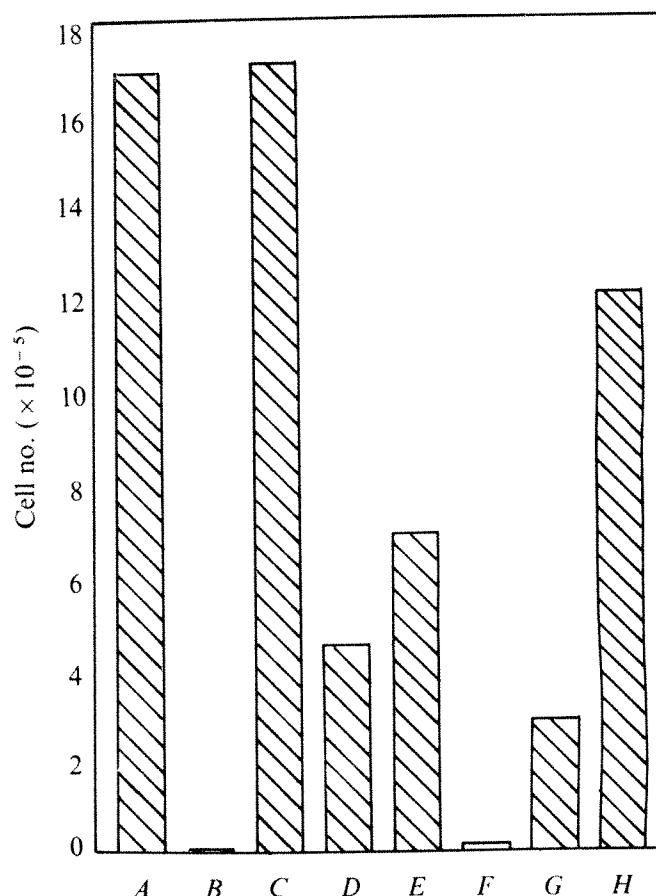
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## Replacement of serum by hormones permits growth of cells in a defined medium

MOST cell cultures require the addition of serum to synthetic media for their maintenance and growth, and we believe that the primary role of the serum is to provide hormones<sup>1</sup>. We have been led to this hypothesis by a series of experiments showing that serum depleted of certain hormones no longer supports growth of cells, unless the medium is supplemented with the hormones that were removed<sup>2–4</sup>. Clear evidence for the validity of this hypothesis has not yet been obtained because it is difficult to grow cells in the absence of serum. Recently, however, we have succeeded in growing an established rat pituitary cell line, GH<sub>3</sub>, in a defined serum-free medium supplemented with physiological concentrations of four hormones together with the iron transport protein, transferrin. Preliminary investigation shows that serum-free medium supplemented with hormone will also support the growth of several other cell lines.

GH<sub>3</sub> is a functional rat pituitary cell line which produces growth hormone and prolactin<sup>5,6</sup>, and depends on thyroid hormones for growth<sup>1</sup>. Initially, the basal medium used in our growth experiments consisted of 8% charcoal-extracted foetal calf serum. At this concentration of serum, the stimulation by triiodothyronine (T<sub>3</sub>) of the growth of these cells was readily demonstrated. As the serum concentration was decreased in a stepwise manner, we were able to find which additional hormones were required; eventually it was

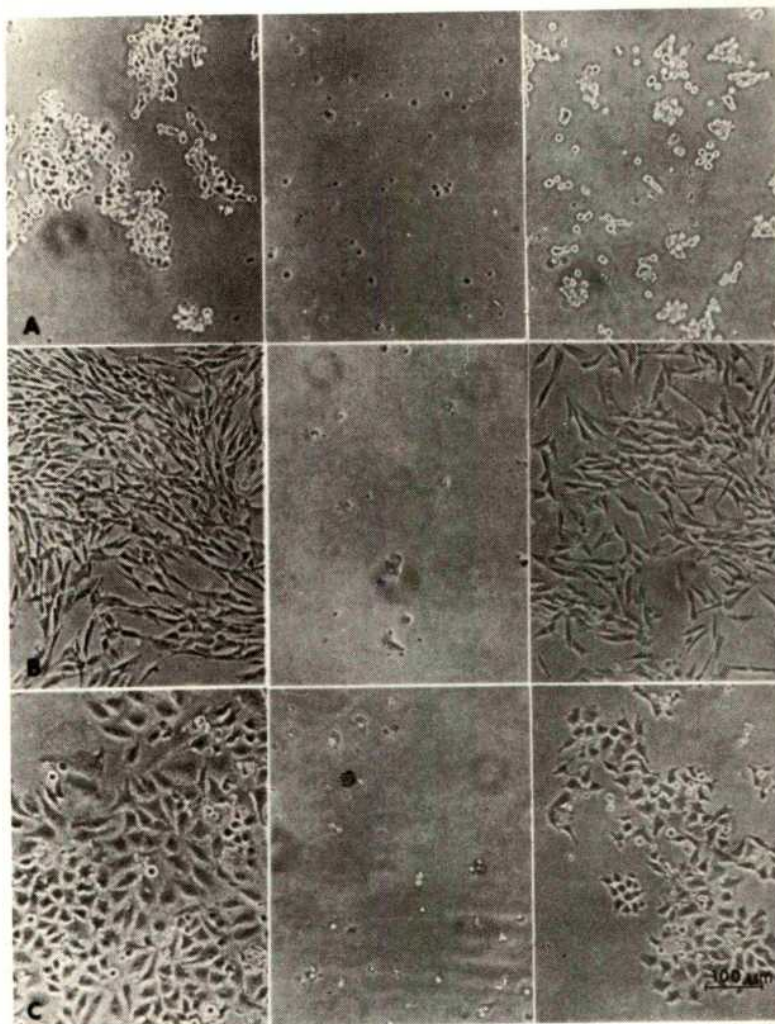


**Fig. 1** GH<sub>3</sub> cells after 10 d of culture in F-12 medium supplemented with 8% foetal calf serum (8% FCS F-12), serum-free F-12 medium (F-12 SF), and F-12 SF supplemented with hormones. The cells from stock plates (in Dulbecco's modified Eagle's medium supplemented with 12.5% horse serum and 2.5% foetal calf serum) were trypsinised into single cells, treated with trypsin inhibitor, suspended in F-12 SF, and then inoculated at  $1 \times 10^5$  cells per 60-mm plate. The hormones were added at the time of plating. The final concentrations of hormones were  $3 \times 10^{-10}$  M T<sub>3</sub>,  $1 \times 10^{-8}$  M TRH, 50 mU ml<sup>-1</sup> somatomedin preparation, 5  $\mu$ g ml<sup>-1</sup> transferrin and 0.5 ng ml<sup>-1</sup> PTH. At the end of the experiments, both the floating and the attached cells were collected by trypsinisation and centrifugation, treated with Trypan blue, and the live cells counted by haemocytometer. A, 8% FCS F-12; B, F-12 SF; C, F-12 SF + hormones (T<sub>3</sub> + TRH + somatomedin A + transferrin + PTH); D, F-12 SF + hormones minus T<sub>3</sub>; E, F-12 SF + hormones minus TRH; F, F-12 SF + hormones minus somatomedin A; G, F-12 SF + hormones minus transferrin; H, F-12 SF + hormones minus PTH. The first-day cell counts for A and C were identical ( $0.9 \times 10^5$  cells per plate). T<sub>3</sub> and transferrin (human) were obtained from Sigma, TRH is a synthetic peptide and PTH is the synthetic peptide of the first 30 amino acids of human PTH.

found possible to eliminate serum entirely if the medium was supplemented with T<sub>3</sub>, thyrotropin-releasing hormone (TRH), transferrin, the biologically active peptide of parathyroid hormone (PTH), and a partially purified somatomedin preparation (5,000-fold purification from serum)<sup>7</sup>. The effect of these hormones on cell growth is shown in Figs 1 and 2. In the control plates, where neither serum nor hormones were added, there were no live cells after 3 d of incubation. When the five components are present in the serum-free medium, the growth is ~60–100% that in the rich medium (8% foetal calf serum in F-12). If any of the components is not added to the medium, growth either does not occur or is severely depressed. This deficiency is not overcome by substituting other pituitary hormones, steroids, hypothalamic releasing hormones, caeruloplasmin, insulin, glucagon, glycyl-histidyl-lysyl acetate, calcitonin, or prostaglandin E<sub>1</sub>.

The results obtained with GH<sub>3</sub> cells encouraged us to

**Fig. 2** Three cell lines in various media after 7 d in culture. Cells: A, GH<sub>3</sub>; B, BHK; C, HeLa. Media from left to right, 8% FCS F-12, F-12 SF, F-12 SF supplemented with hormones. Hormone additions to GH<sub>3</sub> and BHK are described in the text and in Fig. 3. The final concentrations of hormones added to HeLa cultures were:  $1 \times 10^{-9}$  M TRH,  $10 \text{ ng ml}^{-1}$  SRIF and LRH,  $2 \times 10^{-9}$  M hydrocortisone acetate,  $17 \beta$ -oestradiol and testosterone,  $2 \times 10^{-8}$  M progesterone,  $3 \times 10^{-10}$  M T<sub>3</sub>,  $5 \mu\text{g ml}^{-1}$  caeruloplasmin and transferrin,  $0.2 \mu\text{g ml}^{-1}$  glycyl-histidyl-lysyl acetate,  $50 \text{ mU ml}^{-1}$  somatomedin A,  $50 \text{ ng ml}^{-1}$  insulin and glucagon  $0.5 \text{ ng ml}^{-1}$  PTH and  $0.1 \text{ ng ml}^{-1}$  calcitonin.



study the growth of other established cell culture lines in a defined serum-free medium supplemented with hormones. Initial experiments showed that BHK and HeLa cells do not survive in serum-free medium, but can grow in serum-free medium supplemented with a mixture of 25 hormones. The growth in the latter medium is comparable to that in the rich medium. The growth response of these cells to hormones is shown in Figs 2 and 3. Determinations of the specific hormones which support the growth of these cell lines is in progress.

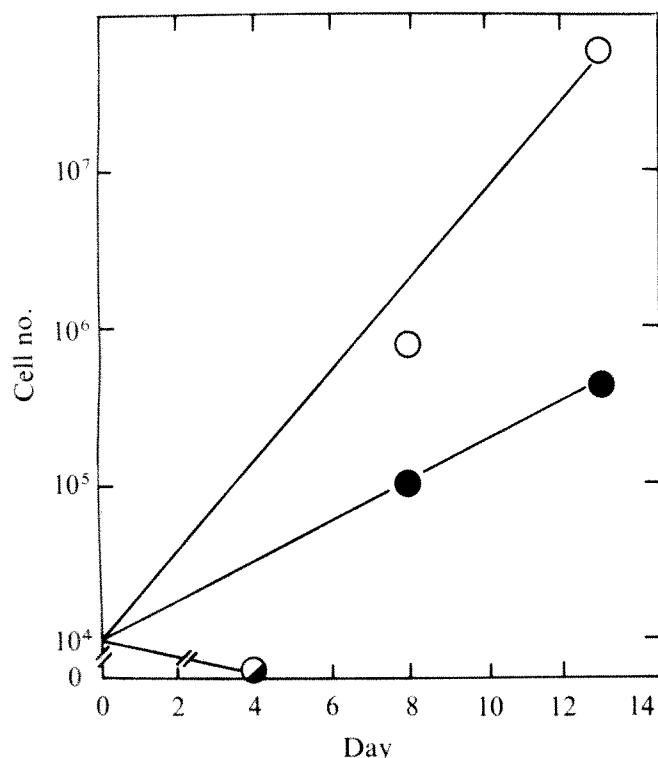
There have been several reports of the growth of cells in completely defined medium<sup>8-10</sup>. Most if not all of these cases are, however, examples of selection or adaptation of cells to defined culture media. By contrast, our data show that the medium supplemented with hormone substitutes for serum without altering the characteristics of the individual cells or the overall population. Our experiments indicate that a combination of hormones and other specific factors, such as transferrin, can substitute completely for serum. Though the possibility of serial propagation of GH<sub>3</sub> is still under investigation, the results obtained with BHK (Fig. 3) strongly suggest that serum-free medium supplemented with hormones will also support the long term growth of GH<sub>3</sub> cells in a completely defined medium. It has already been shown that hormones can partially substitute for some functions of serum such as the overcoming of density-inhibited growth on changing from serum-containing to serum-free medium<sup>11</sup>.

In these experiments, there is a possibility that residual serum factors are still present and active even after extensive washing. We eliminate this possibility in our experiments in which the cells are transferred from serum-containing medium by trypsinisation into defined medium,

and the growth studied entirely in the defined, serum-free medium. It is important to note that there is no delay in the initiation of growth, suggesting that there is neither selection of cells capable of growth in the serum-free medium, nor extensive adaptation to the growth conditions. Furthermore, growth, in the case of GH<sub>3</sub> cells, is not a nonspecific response to the addition of protein to the serum-free medium. The total amount of protein added (transferrin and the somatomedin preparation) is about  $8 \mu\text{g ml}^{-1}$ . Serum protein equivalent to this protein concentration (0.016% foetal calf serum), when added to the serum-free medium, does not support the growth of these cells, even when supplemented with T<sub>3</sub>. We attribute the growth-promoting activity of the transferrin and somatomedin preparations to these two specific factors, although we recognise that other components within these preparations might also be responsible for stimulation of growth. We are continuing to investigate this as more highly purified preparations become available.

The results presented here strongly suggest that it is possible to eliminate serum from culture medium, and that the main function of serum in cell culture is to furnish hormones. We expect that any cell culture line showing a requirement for serum can be grown in synthetic medium supplemented with a combination of hormones and a few factors such as transferrin. Comparison of the hormonal requirements of GH<sub>3</sub>, BHK and HeLa already indicates that the specific hormones will vary with the cell type, although a few hormones may be common to all cell types. We believe that the elucidation of these requirements will be useful in advancing our understanding of integrated physiology. The possibility of culturing cells in serum-free medium supplemented with hormones will enhance the importance





**Fig. 3** Growth of BHK cells in 10% FCS F-12 and in F-12 SF supplemented with hormones. There were no live cells in F-12 SF only, 4 d after the initiation of culture. The initial plating procedure is as described in Fig. 1. The cells were inoculated at  $1 \times 10^4$  cells per 35-mm plate, and the medium was changed on day 5. The cells, both in the rich medium and in the serum-free medium supplemented with hormones were subcultured on day 8 into the respective media. For subcultures, both trypsin and trypsin inhibitor were also used. On day 8 and on day 13, the cells were washed with buffer, trypsinised, and counted with a Coulter counter. The final concentrations of hormones and factors used were:  $0.5 \mu\text{g ml}^{-1}$  LH,  $10 \text{ mU ml}^{-1}$  FSH,  $5 \mu\text{U ml}^{-1}$  ACTH,  $5 \text{ ng ml}^{-1}$  GH,  $10 \text{ ng ml}^{-1}$  prolactin,  $5 \text{ ng ml}^{-1}$  TSH,  $10 \text{ ng ml}^{-1}$  prostaglandin  $E_1$ ,  $5 \mu\text{g ml}^{-1}$  caeruloplasmin and transferrin,  $0.2 \mu\text{g ml}^{-1}$  glycyl-histidyl-lysyl acetate,  $50 \text{ mU ml}^{-1}$  somatomedin A,  $50 \text{ ng ml}^{-1}$  insulin and glucagon,  $0.5 \text{ ng ml}^{-1}$  PTH,  $0.1 \text{ ng ml}^{-1}$  calcitonin,  $1 \times 10^{-9} \text{ M}$  TRH,  $10 \text{ ng ml}^{-1}$  SRIF (somatotropin releasing inhibitory factor) and LRH (LH releasing hormone). ○, 8% FCS F-12; ●, F-12 SF; ●, F-12 SF supplemented with hormones. LH, FSH, GLH, prolactin (bovine) and TSH were supplied by NIAMD Program of National Institutes of Health; ACTH was obtained from Armour Pharmaceutical Co., Chicago Illinois; caeruloplasmin, transferrin and insulin were from Sigma (Bovine) and glycyl-histidyl-lysyl acetate and glucagon were from Calbiochem. Prostaglandin  $E$  was from Dr J. Pike, Upjohn Co.

of cell culture as an experimental tool, especially in overcoming the present difficulties associated with obtaining primary cultures.

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## Interaction of multiplication-stimulating activity with chick embryo fibroblasts demonstrates a growth receptor

MULTIPLICATION-STIMULATING activity (MSA), which is the name given to a family of polypeptides isolated from calf serum<sup>1</sup> and from medium conditioned by certain rat liver cell cultures<sup>2</sup>, stimulates cellular DNA synthesis and growth, and has insulin-like activity. MSA is acid soluble, heat stable and has a molecular weight of approximately 10,000<sup>1,2</sup>. It shares these physical and biological properties with somatomedin<sup>3</sup> and acid-ethanol soluble non-suppressible insulin-like activity (NSILA-s)<sup>4</sup>—polypeptides in human serum which may be important in human growth. We have now studied the interaction of MSA and insulin with chick embryo fibroblasts (CEF). We describe here the binding of labelled MSA to CEF, and correlate this binding with the stimulation of incorporation of <sup>3</sup>H-thymidine into DNA. We propose that MSA and insulin stimulate DNA synthesis in CEF by interacting with a common growth receptor.

MSA was purified from medium conditioned by a cloned line of rat liver cells, BRL-3A<sup>2</sup>, originated by Dr H. Coon. The medium was clarified by centrifugation, chromatographed on Dowex 50 (ref. 2) and twice gel-filtered on Sephadex G-50 in 1 N acetic acid. Fractions which gave a single protein band on disc acrylamide gel electrophoresis at pH 2.7 in 9.0 M urea were used (unpublished results of S.P.N., J. Passamani and P. Short).

Both MSA and insulin<sup>5</sup> stimulated the incorporation of <sup>3</sup>H-thymidine into DNA in tertiary cultures of CEF. The dose-response curves of the two polypeptides were essentially indistinguishable, as were the maximum responses (Fig. 1). Half-maximal stimulation was observed with MSA at  $30 \text{ ng ml}^{-1}$  and insulin at  $45 \text{ ng ml}^{-1}$ . Similar results were reported by Smith and Temin<sup>7</sup>.

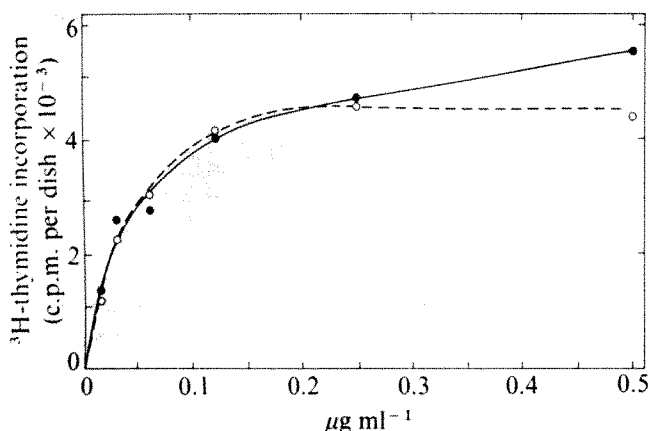
When CEF were incubated concurrently with MSA at  $0.5 \mu\text{g ml}^{-1}$ , and insulin at  $0.5 \mu\text{g ml}^{-1}$ —concentrations which separately gave maximum stimulation of <sup>3</sup>H-thymidine incorporation—the stimulation observed was not

**Table 1** Effect of combined addition of MSA and insulin on <sup>3</sup>H-thymidine incorporation in CEF

Addition	<sup>3</sup> H-thymidine incorporation (c.p.m. per dish $\times 10^{-3}$ )
(1) Control	5.7 $\pm$ 0.1
(2) MSA, $0.5 \mu\text{g ml}^{-1}$	21.0 $\pm$ 0.1
(3) MSA, $1.0 \mu\text{g ml}^{-1}$	19.8 $\pm$ 1.4
(4) Insulin, $0.5 \mu\text{g ml}^{-1}$	14.5 $\pm$ 1.2
(5) Insulin, $1.0 \mu\text{g ml}^{-1}$	17.2 $\pm$ 0.3
(6) MSA, $0.5 \mu\text{g ml}^{-1}$ + Insulin, $0.5 \mu\text{g ml}^{-1}$	15.9 $\pm$ 0.2*
(7) 10% Serum	58.9 $\pm$ 2.7

CEF were incubated for 12 h with the additions shown, pulsed for 1 h with <sup>3</sup>H-thymidine, and the radioactivity incorporated into DNA was determined. The mean and range of duplicate determinations are shown. The data obtained with insulin and MSA ( $0.5 \mu\text{g ml}^{-1}$  and  $1.0 \mu\text{g ml}^{-1}$ ) confirmed that  $0.5 \mu\text{g ml}^{-1}$  was a maximally effective concentration.

\*If the effects of MSA and insulin were additive, the incorporation expected would be 29.8.



**Fig. 1** Stimulation of  $^3\text{H}$ -thymidine incorporation in CEF by MSA and insulin. Tertiary cultures of CEF were plated in 0.4% ET (Temin's modified Eagle's medium with 20% by volume tryptose phosphate broth and 0.4% foetal calf serum)<sup>3</sup>. Three days later, insulin (crystalline porcine, Eli Lilly), MSA or ET medium were added at the concentrations indicated. After incubation for 12 h, the cultures were pulsed for 1 h with  $^3\text{H}$ -thymidine, 0.2  $\mu\text{Ci ml}^{-1}$ , and the incorporation of radioactivity into acid-precipitable material (DNA) was determined<sup>6</sup>. The c.p.m. incorporated per 60-mm Petri dish are plotted. The points are the average of duplicate determinations. The incorporation, 2,830 c.p.m., in a control dish which received medium alone, has been subtracted. The MSA and insulin dose-response curves presented are representative. In general, however, the maximal stimulation by MSA (○) is slightly greater than that obtained with insulin (●).

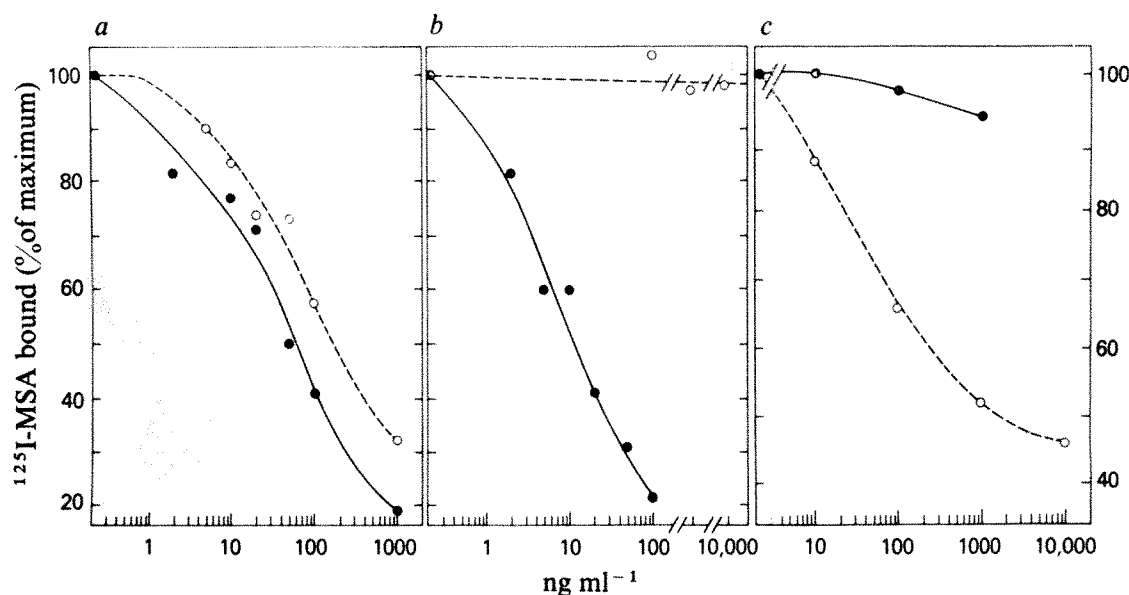
the sum of individual stimulations (Table 1). Since foetal calf serum induced a greater rate of  $^3\text{H}$ -thymidine incorporation, the non-additive effects of insulin and MSA suggested that the two peptides act by a common mechanism. Non-additivity has been observed with insulin and NSILA-s<sup>8</sup>, and insulin and MSA (unpublished results) in human fibroblasts, and with insulin and MSA in 3T3 mouse fibroblasts<sup>9</sup>. In contrast, insulin, MSA and NSILA-s gave

additive responses with serum and fibroblast growth factor in these systems<sup>8,9</sup>.

Polypeptide hormones such as insulin and MSA exert their biological effects by interacting with specific plasma membrane receptors<sup>10</sup>. The apparent common pathway for the stimulation of DNA synthesis in CEF by MSA and insulin could be explained if they utilised the same cell surface receptor. To examine this possibility, MSA was radioactively by the chloramine T procedure as modified by Megyesi *et al.*<sup>11</sup> and the binding of  $^{125}\text{I}$ -MSA to CEF studied.

$^{125}\text{I}$ -MSA bound rapidly and reversibly to CEF at 22 °C. Half the maximal binding was achieved in 45 min, and a plateau of binding was maintained for 3–6 h. Approximately 1–4% of the tracer MSA was bound per  $10^6$  CEF. The bound radioactivity was reduced by more than 70% when incubation was conducted in the presence of an excess of unlabelled MSA ( $1 \mu\text{g ml}^{-1}$ ). Competitive binding with different concentrations of unlabelled MSA gave a steep displacement curve (Fig. 2a). Binding was inhibited 50% by MSA at  $50 \text{ ng ml}^{-1}$  in this experiment, and at  $33 \pm 5 \text{ ng ml}^{-1}$  in 13 experiments. Unrelated peptides failed to inhibit the binding of MSA tracer even in high concentrations: human growth hormone, glucagon and nerve growth factor (A. Liuzzi) at  $10 \mu\text{g ml}^{-1}$ ; epidermal growth factor (R. Ledda) at  $600 \text{ ng ml}^{-1}$ ; and fibroblast growth factor (D. Gospodarowicz) at  $200 \text{ ng ml}^{-1}$ . In contrast, in seven experiments insulin was  $66 \pm 13\%$  as potent as MSA by weight in competing for MSA tracer binding (Fig. 2a).

The near equivalence of MSA and insulin in competing for the binding of MSA to CEF differs strikingly from their relative potencies in other binding systems. In rat liver membranes, for example, MSA is more than four orders of magnitude more effective than insulin in competing for  $^{125}\text{I}$ -MSA binding (Fig. 2b). In cultured human lymphocytes, on the other hand, insulin competes for the binding of MSA tracer appreciably better than does MSA itself (Fig. 2c). This suggests that lymphocytes lack an MSA



**Fig. 2** Binding of  $^{125}\text{I}$ -MSA to CEF rat liver membranes and cultured human lymphocytes. MSA was iodinated with  $\text{Na}^{125}\text{I}$  to a specific activity of  $150 \text{ Ci g}^{-1}$  by a modification of the chloramine T procedure<sup>11</sup>.  $^{125}\text{I}$ -MSA gave a single sharp peak on Sephadex G-50 gel filtration in 1 N acetic acid and co-migrated with unlabelled MSA on disc acrylamide electrophoresis. Tertiary cultures of CEF were detached from the cell monolayer by gentle trypsinisation (0.05% trypsin 0.5 mM EDTA, 37 °C, 5 min). Binding experiments were performed under conditions chosen to give a steady state. *a*,  $^{125}\text{I}$ -MSA, 500  $\text{pg ml}^{-1}$ , was incubated in HEPES binding buffer<sup>12</sup>, pH 8.0, in a total volume of 0.5 ml, with  $2 \times 10^6$  CEF for 3 h at 22 °C; *b*,  $^{125}\text{I}$ -MSA (500  $\text{pg ml}^{-1}$ ) was incubated with purified rat liver plasma membranes (kindly given by D. M. Neville Jr) in 0.15 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 1% bovine serum albumin for 90 min at 22 °C; *c*,  $^{125}\text{I}$ -MSA (500  $\text{pg ml}^{-1}$ ) was incubated with  $8 \times 10^6$  cultured human lymphocytes (line IM-9) in 0.5 ml of HEPES binding buffer, pH 8.0, for 90 min at 15 °C. The indicated concentrations of unlabelled MSA (●) and insulin (○) were added together with tracer MSA and were present throughout the incubation. In each experiment, cells or membranes are pelleted by centrifugation for 1 min in a Beckman Microfuge, the pellets were excised and the bound radioactivity was determined. The total binding was 48%, 3.7% and 6.2% of the tracer radioactivity in the three experiments, respectively. Maximum binding has been plotted at 100%.



receptor, tracer MSA binding exclusively to lymphocyte insulin receptors. The poor competition by unlabelled MSA is consistent with its low insulin-like activity (<1% the potency of insulin). Analogous results have been reported for NSILA-s binding to rat liver membranes and cultured lymphocytes<sup>11</sup>.

CEF have insulin receptors (our unpublished results) similar to those in other tissues<sup>13,14</sup>. The MSA binding we have observed, however, clearly is not binding to an insulin receptor. <sup>125</sup>I-insulin binding to insulin receptors is inhibited by different peptides in proportion to their insulin-like biological activities. MSA and NSILA-s, which have low insulin-like activity, compete poorly for the binding of insulin tracer. In contrast, they are more than 250 times more potent competitors for MSA binding than for insulin binding in CEF (our unpublished results). Porcine proinsulin is 20 times more potent in competing for MSA binding than for insulin binding (our unpublished results). Scatchard plots of insulin binding data are characteristically curved<sup>15</sup>. This results from negatively cooperative site-site interactions among insulin receptors<sup>15</sup>. In contrast, Scatchard plots of MSA-binding data are linear (our unpublished results). We have been unable to demonstrate cooperative interactions among MSA receptors.

In summary, CEF possess MSA receptors with a unique specificity, that is, insulin competes almost as well for tracer MSA binding as MSA itself. The CEF MSA receptors differ from insulin receptors, MSA receptors of rat liver membranes, and NSILA-s receptors in CEF<sup>16</sup>. MSA and insulin stimulate DNA synthesis in CEF with nearly identical dose-response curves. Their effects are not additive. The same MSA concentration induces half-maximal <sup>3</sup>H-thymidine incorporation and inhibits tracer MSA binding by 50%. MSA preparations at different stages of purification and inactivated MSA compete for tracer MSA binding in proportion to their potencies in stimulating <sup>3</sup>H-thymidine incorporation (our manuscript in preparation). Taken together, these properties suggest that the MSA receptor we have demonstrated in CEF is a physiologically relevant growth receptor, mediating the growth-promoting effects of MSA and insulin.

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## Two sex steroid receptors in mouse fibroblasts in culture

WE report that the mouse fibroblasts known as L-929 cells have androgen and oestrogen receptors (R-A and R-E), as well as the glucocorticosteroid receptors (R-G) reported earlier<sup>1,2</sup>. This observation may lead to an increased interest in L cells as they provide a convenient system for studies of the biology and pharmacology of hormones at the cellular level.

L-929 cells provided by J. Zeuthen, Institute for Human Genetics, Aarhus, who obtained them from the American Type Culture Collection, and found that they had 56-76 chromosomes, with a modal number of 66 (personal communication). Cells were grown in plastic Petri dishes, or in suspension using Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated calf serum, bovine insulin (0.12 U ml<sup>-1</sup>) and antibiotics. At confluence, cells were collected by scraping the dishes with a rubber policeman, or by low speed centrifugation from suspension. After washing in cold phosphate-buffered saline, cells were homogenised in a small volume of TET-buffer (Tris 15 mM,

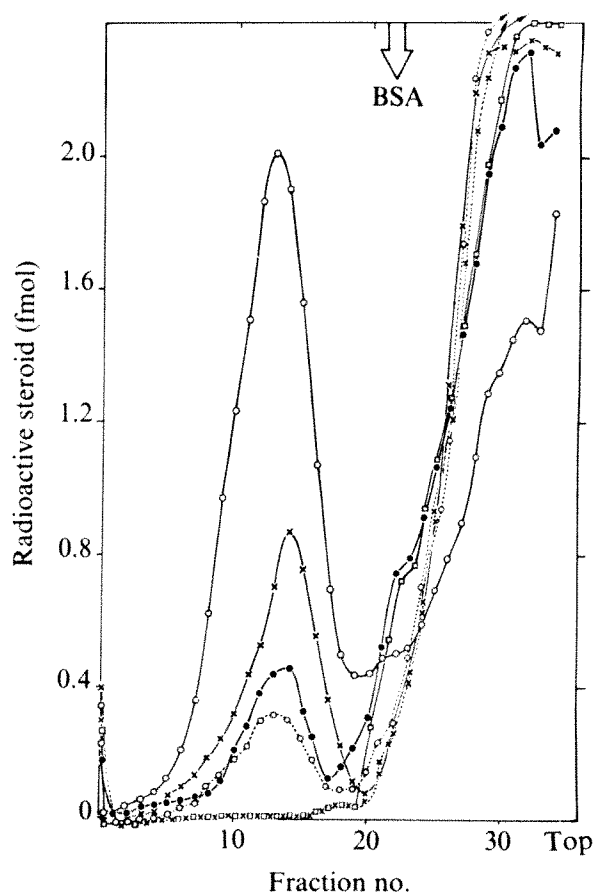
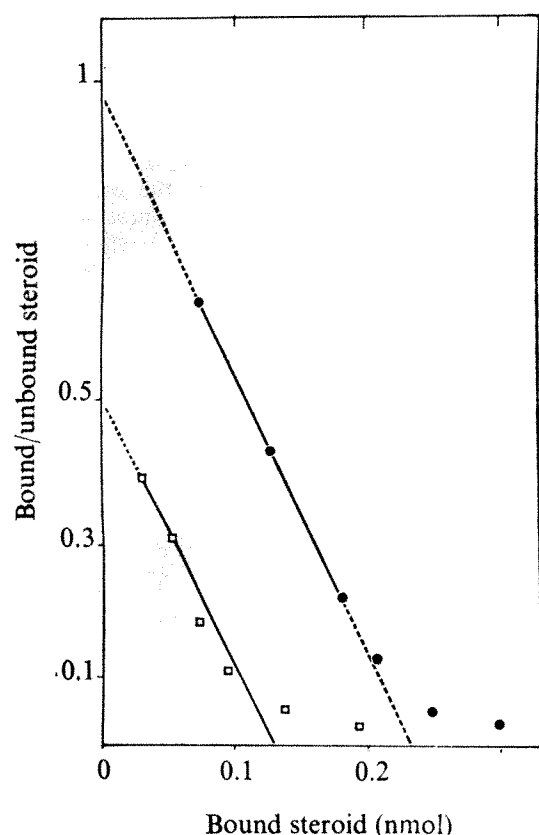


Fig. 1 Ultracentrifugation of L-929 cell cytosol preincubated with radioactive dexamethasone, androstanolone and oestradiol on linear 5-35% glycerol density gradients in TET buffer. Cytosols were labelled at 0-2 °C for 3 h and 0.3 ml samples were centrifuged at 149,000g<sub>av</sub> for 18 h at 0-2 °C. Thirty-four fractions (2 drops) were collected (abscissa), counted and the results expressed in fmol of radioactive steroid (ordinate). ○—○, 1 nM <sup>3</sup>H-dexamethasone; ○—○, 1 nM <sup>3</sup>H-dexamethasone plus 100 nM unlabelled dexamethasone; ×—×, 1 nM <sup>3</sup>H-androstanolone; ×—×, 1 nM <sup>3</sup>H-androstanolone plus 100 nM unlabelled androstanolone; ●—●, 1 nM <sup>3</sup>H-oestradiol; □—□, 1 nM <sup>3</sup>H-oestradiol plus 100 nM unlabelled oestradiol.



**Fig. 2** Scatchard plot of binding data for androstanolone and oestradiol by L-929 cell cytosol. Binding of  $^3\text{H}$ -androstanolone ( $\bullet$ ) and  $^3\text{H}$ -oestradiol ( $\square$ ) in the 105,000g cytosol (4.9 mg protein per ml) from L cells, using increasing concentrations of steroid from 0.2 nM to 100 nM. Specific binding was determined after exposure to charcoal-dextran (5.0–0.5 mg ml $^{-1}$  TET buffer) at 0 °C for 30 min followed by centrifugation and counting of the supernatant.

EDTA 1 mM, dithiothreitol 1 mM, pH 7.4) in a glass-glass homogeniser. The cytosol fraction was obtained after alternate centrifugations at 700g for 15 min and 105,000g for 60 min. Looking for the cytosol receptor, we used purified  $^3\text{H}$ -steroids: androstanolone (5 $\alpha$ -androstane-17 $\beta$ -ol-3-one; 175 Ci mmol $^{-1}$ ), dexamethasone (9 $\alpha$ -fluoro-16 $\alpha$ -methyl-11 $\beta$ , 17,21-trihydroxypregn-1,4-diene-3,20-dione; 27 Ci mmol $^{-1}$ ), 17 $\beta$ -oestradiol (60 Ci mmol $^{-1}$ ), progesterone (48 Ci mmol $^{-1}$ ) and R5020 (17,21-dimethyl-19-nor-4,9-pregnandiene-3, 20-dione; 51.4 Ci mmol $^{-1}$ ). Binding was achieved after incubation at 0–2 °C for at least 2 h, mostly at 1 nM. Unlabelled compounds were used in isotopic dilution experiments to assess nonspecific binding by a subtraction method or to measure binding inhibition, and to calculate

the number of binding sites and the  $k_{\text{deg}}$  for a series of measurements made with different concentrations of steroids. Binding was evaluated either by using a charcoal suspension to remove free and rapidly released steroids, or by ultracentrifugation through linear 5–35% glycerol Tris gradients as described before<sup>4–6</sup>.

A symmetrical radioactive 7–8S peak was obtained after labelling with 1 nM  $^3\text{H}$ -androstanolone,  $^3\text{H}$ -oestradiol or  $^3\text{H}$ -dexamethasone. No binding was observed after incubation with progesterone or R5020, a progestational agent of high affinity for progesterone receptor in the uterus and mammary gland. As Fig. 1 shows, the radioactive 7–8S peak of R-G was greatest, while that of R-A was twice as high as the R-E peak. Addition of the corresponding unlabelled steroid (100 nM) to the 1 nM  $^3\text{H}$ -tracer almost abolished the radioactive 7–8S peaks of R-A and R-E, while 15% of R-G binding remained in the 7–8S area. In the 4S region of the gradient, no binding was observed with  $^3\text{H}$ -androstanolone but some nonspecific binding (not depressible by isotopic dilution) was seen with  $^3\text{H}$ -oestradiol and  $^3\text{H}$ -dexamethasone. Treatment with Pronase (1 mg ml $^{-1}$ ) for 2 h at 0 °C abolished the 7–8S peak of 1 nM  $^3\text{H}$ -androstanolone and  $^3\text{H}$ -oestradiol in the cytosol.

After incubation with various concentrations of steroid (0.25–100 nM), the Scatchard plot of the data (Fig. 2) indicated  $k_D \sim 0.25$  nM for androstanolone and 0.20 nM for oestradiol with 47.8 and 23.5 fmol mg $^{-1}$  protein of cytosol (measured by the Lowry method), respectively, corresponding to about 3,000 and 1,500 sites per cell (calculated from the cell number).

Specificity studies by competition assays with unlabelled steroids (100 nM, Table 1) suggested that the three binding sites are probably associated with three distinct and non-interacting sites, and that they are similar to the oestrogen, androgen and glucocorticosteroid receptors described in many mammalian target organs<sup>4–6</sup>. Oestrogens such as the non-steroid diethylstilboestrol (DES) compete with  $^3\text{H}$ -oestradiol for receptors, but cortisol, progesterone and androgens do not interfere significantly with the binding of oestradiol.  $^3\text{H}$ -androstanolone binding is inhibited by androstanolone and testosterone, and to a smaller extent by progesterone and cyproterone acetate, an anti-androgen (of the progesterone series), and by oestradiol, but neither DES nor cortisol competes significantly. These results cannot be explained by the presence of a single binding site, but they are compatible with two sites, R-A and R-E. The same strict oestrogen specificity for R-E has been observed in all oestrogen sensitive tissues studied so far, while competition by androgens, progesterone, anti-androgens and oestradiol, but not DES, has also been observed with all androgen receptors<sup>3,7–10</sup>.

Furthermore, the small degree of competition between  $^3\text{H}$ -dexamethasone and androstanolone or oestradiol, and conversely the virtual absence of competition on R-A and

**Table 1** Specificity studies for steroid receptors in L-929 cells

Radioactive hormone (1 nM)	R-A $^3\text{H}$ -androstanolone	R-E $^3\text{H}$ -oestradiol	R-G $^3\text{H}$ -dexamethasone
No competitor*	100	100	100
Non-radioactive competitor (100 nM)			
Androstanolone	13	91	90
Testosterone	16	99	—
Cyproterone acetate	29	98	80
Oestradiol	47	16	90
DES	95	20	93
Progesterone	39	97	33
Cortisol	92	92	34
Dexamethasone	—	—	22

Samples of cytosol were incubated at 0–2 °C with  $^3\text{H}$ -steroids with or without unlabelled steroids for 3 h, and bound radioactivity was obtained after 30 min of exposure to charcoal-dextran (as described in Fig. 2).

\*100 expresses the binding with 1 nM  $^3\text{H}$ -steroid and corresponds to 18.0 fmol bound androstanolone, 9.4 fmol bound oestradiol and 40.0 fmol bound dexamethasone per mg cytosol protein, respectively, for R-A, R-E and R-G.

R-E binding by cortisol favour independent R-G binding sites. No progesterone receptor was detected.

To investigate nuclear receptors, we obtained nuclei from the pellet of the homogenate after centrifugation at 700g for 15 min. Nuclei were suspended in 0.25 M sucrose, 1 mM MgCl<sub>2</sub> and 10 mM Tris buffer, pH 7.5, containing 1% Triton and washed three times in the same buffer without Triton. We looked for the nuclear receptor by incubating the nuclear suspension directly, or after labelling the 0.5 M KCl nuclear extract. In the first case, nuclei were resuspended in sucrose buffer and samples were incubated with 1 nM or 5 nM tritiated steroids, alone or with a 100-fold excess of the corresponding unlabelled compound for 1 h at 0 °C followed by 2 h at 20 °C. After washing three times with excess buffer followed by methanol extraction of the pellet, no specific binding was observed. By the other approach, the washed nuclear pellet was extracted with high salt buffer (TET-0.5 M KCl), centrifuged at 105,000g for 60 min and the soluble extract was incubated with 1 nM or 5 nM tritiated hormones and tested for binding by charcoal-dextran or density gradient centrifugations. No specific binding could be observed as in target tissue nuclei from hormone-deprived animals<sup>4-6</sup>. This was compatible with the presence of a very small or insignificant amount of steroid in the medium (verified by direct radio-immunoassays), and facilitated further study of hormonal effects on receptor distribution.

Cells were incubated for 30 or 60 min with 5 nM <sup>3</sup>H-androstanolone or <sup>3</sup>H-oestradiol at 37 °C in 5% CO<sub>2</sub> in air. A 5.3S peak was obtained in the 0.5 M KCl extract after gradient ultracentrifugation analysis, corresponding in the case of <sup>3</sup>H-androstanolone to 20.6 fmol mg<sup>-1</sup> protein after 30 min, and 23.7 fmol mg<sup>-1</sup> after 60 min of incubation. In the cytosols, analysed on glycerol-Tris gradients, 4.6 fmol mg<sup>-1</sup> protein remained in the 7-8S peak after 30 min and 3.6 fmol mg<sup>-1</sup> after 60 min. After 60 min of incubation with <sup>3</sup>H-oestradiol, the 5.3S nuclear peak corresponded to a protein concentration of 9.4 fmol mg<sup>-1</sup> and the 7-8S cytosol peak to 1.2 fmol mg<sup>-1</sup>. As in other systems<sup>11</sup>, about 40% of nuclear radioactivity was not extracted by KCl buffer, and was not studied further. When the cells were incubated together in 5 nM <sup>3</sup>H tracer and 500 nM unlabelled steroid, no 5.3S peak was observed in the nuclear extract.

These results suggested that, as in all steroid-sensitive cells the cytosol receptors in L-929 cells are transferred into the nuclei in the form of <sup>3</sup>H-steroid-receptor complexes after brief exposure of L cells to the appropriate hormone at 37 °C.

As the three cytosol receptors had the same S value on gradient ultracentrifugation, we investigated whether two or three of the different binding sites could belong to the same macromolecule. We reasoned that, if this is the case, the transfer of the oestradiol receptor into the nucleus would leave available androgen binding sites in the soluble nuclear extract (and vice versa for the transfer of R-A). But no binding was observed, after exposure of cells to 5 nM unlabelled oestradiol at 37 °C and secondary labelling of the nuclear 0.5 M KCl extract for 2 h at 0 °C with 1 nM <sup>3</sup>H-androstanolone. Similar results were obtained with incubation in other steroids followed by hetero-labelling and all results were negative.

Therefore, we conclude that R-A, R-E and R-G are distinct binding molecules, as already observed for R-A and R-E in MI<sub>1</sub> cells<sup>3</sup> and in the uterus and prostate<sup>12,13</sup>. These data for cell lines such as MI<sub>1</sub> or L suggest that different receptors are present simultaneously in a single cell, an impossible conclusion for organs made up of various cell types. In the case of the uterus, however, the induction of oestradiol and progesterone receptors by progesterone and oestradiol, respectively<sup>14-16</sup>, favours the likelihood of a cellular multiplicity of types of receptor within a cell.

Thus by all criteria of binding affinity and specificity, physicochemical properties, cell distribution and nuclear transfer L-929 cells contain three typical steroid receptors, very similar to functional target organ receptors detected already. This supports the concept of cellular plurality of receptors, which in turn should be studied in terms of potentially different interactions with the genome<sup>6,17,18</sup>. It is interesting that oestradiol, the native oestrogen, can, in a given cell, interact with two specific proteins, R-E and R-A, of different affinities, and that a synthetic oestrogen, DES of great therapeutic interest<sup>19</sup>, binds strongly to one of them, R-E, and very little to the other, R-A.

Are these receptors vestigial and unable to mediate steroid effects? Our preliminary observations of L-929 cells growing in the presence of steroids show that with 30 nM androstanolone, there is a moderate increase of growth rate. Moreover, after 10 d of suspension culture with this androgen, the amount of cytosol R-E per mg of cytosol protein decreases approximately 50% (which is not explained by an increase of nuclear binding sites). Conversely, after 10 d in 30 nM DES (17 $\beta$ -oestradiol cannot be used because it binds to R-A), there is no decrease in R-A sites in the cytosol. Macromolecular syntheses (total proteins and DNA) are also changed by different steroid concentrations and combinations. Normal fibroblasts (ref. 20 and A. Groyer and P. Robel, unpublished) contain sex steroid receptor(s), and it will be interesting to see if these cells are more endocrinologically competent than previously believed.

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## Hereditary persistence of foetal haemoglobin with $\beta$ -chain synthesis in cis position ( $\gamma$ - $\beta$ <sup>+</sup>-HPFH) in a negro family

HEREDITARY persistence of foetal haemoglobin (HPFH) is an uncommon condition described mainly in negroes and Greeks. Although this genetic disorder is rare, it has been very important in the study of the arrangement of human globin genes on chromosomes and in the investigation of the regulation of foetal haemoglobin synthesis. It is not

always possible to distinguish clearly between HPFH and thalassaemia and there is considerable genetic heterogeneity in these conditions. A majority of patients with HPFH have an increased amount of foetal haemoglobin, normal or near normal red cell indices, Hb F in each red cell, an absence of clinical manifestations in the simple heterozygous state or in combination with Hb S, Hb C, or Hb E, and balanced globin synthesis<sup>1</sup>. Two different forms of  $\gamma$  chains are normally synthesised, with either glycine or alanine at the  $\gamma^{136}$  position. Although the majority of negro heterozygotes for HPFH have both glycine and alanine at  $\gamma^{136}$ , several patients have had only glycine<sup>2-5</sup>. There was no synthesis of  $\beta$  or  $\delta$  chains in the *cis* position to the HPFH gene in any of the appropriate negro cases reported previously. We describe here a negro family in which the proposita had Hb S-HPFH with  $\beta$ -chain synthesis in the *cis* position to the HPFH gene.

In a screening study conducted in high schools in Philadelphia, an asymptomatic female teacher with unusual proportions of Hb S, Hb A, and Hb F was detected by cellulose acetate electrophoresis. The Hb S level was 40.9%, Hb F 29.7%, Hb A 27.5% and Hb A<sub>2</sub> 1.9%. The presence of Hb S was confirmed by the dithionite-phosphate solubility test<sup>6</sup> and by precipitation during shaking of the oxy-form of the haemoglobin<sup>7</sup>. On acid elution of peripheral blood smears<sup>8</sup> all her red cells were found to contain Hb F, but in varying amounts. Further study of the patient revealed that she had never experienced symptoms of sickling, but that her red cell morphology as well as her red cell indices and reticulocyte count were normal. A study of her family (Table 1) showed that her husband, her putative father and her sister were normal, but that her 9-month-old son had an Hb F level of 22.1% and an Hb A<sub>2</sub> of 1.1% with normal red cell indices and Hb F present in every red cell. Her mother had sickle cell trait with Hb S of 40.0%. A study of 12 different blood group systems in the proposita and her parents failed to exclude paternity. Globin synthesis studies were performed on each member of the family (Table 1). The results showed balanced globin synthesis in each individual, a result in agreement with the normal red cell indices in failing to detect evidence of thalassaemia in this family. In the proposita the  $\beta^A/\alpha$  and  $\beta^S/\alpha$  specific activity ratios were near unity (1.06, 1.08), results different from the decrease in one or both of these ratios found in negroes with Hb S- $\beta^+$  thalassaemia<sup>10</sup>. Analysis of the glycine and alanine residues in the third portion of the  $\gamma$  chain after cyanogen bromide cleavage<sup>11</sup> gave values in the proposita of glycine 1.02 and alanine 1.97, and in her son of glycine 1.07 and alanine 2.01. These findings may be interpreted as consistent with production of chains with only glycine at  $\gamma^{136}$ .

The presence of HPFH in the proposita and her son is indicated by Hb F being present in all red cells, normal haematological values, balanced globin synthesis and the lack of interaction with Hb S. This family is of special interest in that  $\beta$ -chain production is present and directed

by the chromosomes which contains the HPFH determinant, in contrast to all other negro varieties previously described. The HPFH gene in the negro family described here differs from the British<sup>12</sup> and Greek<sup>13</sup> types in the higher amount of Hb F produced and in the content of only glycine at  $\gamma^{136}$ . Production of  $\beta^A$  chain by the affected chromosome probably occurs in all three varieties. Although the child of the proposita clearly has HPFH, neither parent of the proposita has this condition. Non-paternity was not demonstrated by the studies performed, but either this or a mutation are possibilities in this family.

The majority of patients with HPFH have balanced globin synthesis, although three negro heterozygotes and one homozygote have had clearly decreased ratios<sup>1,12,14,15</sup>. Although many negro heterozygotes with  $\beta$  thalassaemia have normal globin synthesis ratios<sup>16,17</sup>, the findings in this family are not consistent with the presence of  $\beta$  thalassaemia; nor are the normal globin synthesis studies and haematological values<sup>18</sup>.

Studies involving patients with haemoglobin Kenya have indicated that the genes for  $\gamma$ ,  $\delta$  and  $\beta$  chains are closely linked on the same chromosome<sup>19,20</sup>. In the previously described forms of negro HPFH the findings may be attributed to the deletion of the  $\delta$  and  $\beta$  chain loci, together with some controlling regions which allow continuing synthesis of  $^G\gamma$  and  $^A\gamma$ , or only  $^G\gamma$ . In the present family, the low Hb A<sub>2</sub> values suggest that the  $\delta$ -chain locus may be deleted. The total deletion in this family may therefore involve the  $^A\gamma$  and  $\delta$  loci and some controlling regions, leaving  $^G\gamma$  and  $\beta$  chain synthesis intact. It is of interest that in the proposita the percentage of Hb S is almost identical to that found in her mother and similar to that usually found in sickle cell trait, demonstrating that the combined synthesis of Hb F and Hb A directed by the HPFH chromosome is similar to the synthesis of Hb A seen in sickle cell trait and greater than the synthesis of Hb S. As observed in this patient and in other negro heterozygotes for Hb A-HPFH or Hb S-HPFH, the limit of Hb F synthesis per cell seems to be approximately 30–35%. In patients with Hb S- $\beta^0$ -HPFH, the normal haemoglobin content of the cell is achieved by compensation from the  $\beta^S$  gene, which is responsible for approximately 70% of the total haemoglobin production. In Hb S- $\beta^+$ -HPFH described here, Hb F still comprises only 30% of the total haemoglobin in the cell, although Hb A is now responsible for the compensatory synthesis which results in a normal mean cell haemoglobin value. Another family with similar characteristics has recently been found<sup>21</sup>.

The presence of Hb A in the proposita indicates that genetically directed Hb synthesis may persist in each red cell without loss of the  $\beta$  chain cell locus. The importance of this finding is that a variety of defects seem to control HPFH, suggesting that a search for the biochemical defects in these various conditions may provide clues for influencing the synthesis of Hb F in persons who retain  $\beta$  chain

Table 1 Haematological and globin synthesis data on family members

Subject	Age	Hb (g dl <sup>-1</sup> )	MCV ( $\mu$ m <sup>3</sup> )	MCH (pg)	Phenotype (Hb)	Hb A <sub>2</sub> (%)	Hb F (%)	non- $\alpha/\alpha$
Proposita	27	12.0	92.0	30.6	SFA	1.9	29.7	1.08
Husband	28	15.2	92.0	31.5	A	2.9	0.1	0.92
Son	9/12	11.7	82.0	27.7	AF	1.1	22.1	1.17
Sister	23	12.3	90.0	29.4	A	2.4	0.4	1.06
Mother	56	13.1	88.0	29.7	AS	—	0.3	1.03
Father	67	14.4	88.0	29.7	A	2.9	0.8	0.97

The non- $\alpha/\alpha$  ratio was determined after incubation of peripheral blood with <sup>14</sup>C-leucine for 2 h at 37 °C, preparation of haemolysate and separation of the globin chains by chromatography on carboxymethyl cellulose in 8 M urea at pH 6.5 with a sodium phosphate gradient<sup>9</sup>. The total radioactivity in  $\alpha$ ,  $\beta$ S and  $\beta$ A globin peaks was summed when these chains were present in the sample, and was divided by the  $\alpha$ -globin peak to obtain the non- $\alpha/\alpha$  ratio.



loci, such as those with sickle cell disease and thalassaemia major.

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## Permeability of the blood-cerebrospinal fluid barrier to plasma proteins during foetal and perinatal life

HIGH levels of alpha foetoprotein (AFP), albumin and IgG have been detected in the cerebrospinal fluid (CSF) of six human foetuses between 16.5 and 25 weeks old<sup>1</sup>. The levels of AFP were found to decline from 1,200  $\mu\text{g ml}^{-1}$  in a foetus of 16.5 weeks to 52 and 60  $\mu\text{g ml}^{-1}$  in the older ones tested. During this period of foetal life, the ratios between albumin and AFP in CSF were shown to increase. The levels of IgG ranged between 60 and 117  $\mu\text{g ml}^{-1}$  and were higher than those detected in CSF from normal adult subjects (20-40  $\mu\text{g ml}^{-1}$ )<sup>2</sup>.

These preliminary results have prompted us to extend our investigations to CSF from a larger group of human foetuses and compare the levels of albumin, AFP and IgG with those of total protein, transferrin,  $\beta$ -trace protein<sup>3,4</sup>,  $\beta$ 2-microglobulin<sup>5</sup> and lysozyme<sup>6</sup>. Samples of CSF were obtained from 13 foetuses between 14 and 25 weeks old and one sample from a foetus 40 weeks old with hydrocephaly and severe achondroplasia (Table 1). Some of these foetuses were obtained by hysterotomy and had chromosome abnormalities; others were aborted spontaneously and apparently normal. One was a male foetus obtained by therapeutic abortion from a woman carrier of a gene for haemophilia.

CSF was collected by aspiration with a syringe from the cisterna magna; only CSF samples which were absolutely clear from contamination by blood were tested.

The levels of albumin, transferrin, IgG and  $\beta$ -trace protein were estimated by the single radial diffusion technique<sup>7</sup>; AFP was measured by the one dimensional antigen-antibody electrophoresis (rocket technique)<sup>1</sup>; lysozyme by

the lysoplate method<sup>8</sup> and the levels of  $\beta$ 2-microglobulin by radioimmunoassay (Phadebas,  $\beta$ 2-microtest, Pharmacia).

The levels of AFP were higher in CSF of foetuses less than 20 weeks old than in the older foetuses; in two foetuses 16.5 and 19 weeks old the levels were more than 1  $\text{mg ml}^{-1}$ , whereas in three foetuses 25 weeks old the values of AFP varied between 17 and 52  $\mu\text{g ml}^{-1}$  (Table 1). In the 40-week-old foetus, the concentration of AFP in CSF was less than 1  $\mu\text{g ml}^{-1}$ . The levels of albumin and the ratios of albumin to AFP were higher in foetuses between 20 and 25 weeks old than in the younger foetuses (Table 2). Ratios between 1.8 and 4.7 were observed when the levels of total proteins and those of albumin were compared.

IgG was detected in all foetuses studied and slightly higher levels were observed in samples from foetuses between 20 and 25 weeks old.  $\beta$ 2-Microglobulin was found in all samples tested (Table 1) and the ratio IgG/ $\beta$ 2-microglobulin showed only minor variations, ranging between 48 and 116 (Table 2). Lysozyme could not be detected in the foetal CSF using the lysoplate technique;  $\beta$ -trace protein, was found in all foetal CSF and appeared to be a major protein of this body fluid.

In summary, all the proteins tested, with the exception of lysozyme, have been found in foetal CSF in concentrations much higher than those detected in CSF from normal adult subjects. These results, therefore, support the suggestion that the human blood-CSF barrier is not fully developed during foetal life<sup>1</sup> and explain the detection of the high levels of IgG and the presence of specific maternal antibodies in CSF of human infants tested during the first weeks of life<sup>8-10</sup>.

The presence of high levels of AFP in foetal CSF also supports the view that the increased amount of this foetal protein detected in the amniotic fluids of foetuses with open neural-tube defects is due to the transfer of AFP from CSF into the amniotic cavity<sup>1,11,12</sup>.

In view of the high levels of proteins detected in CSF from human foetuses, the transfer of labelled proteins from the blood to the CSF was investigated in newborn and adult rats (M.A. and S.H.). Albumin and IgG were isolated from adult rat serum by precipitation with ammonium sulphate, DEAE chromatography and gel filtration on Sephadex G-200 (ref. 7). Rat IgG antibodies against brain antigens were isolated, using similar techniques, from an immune serum given by Dr R. Hughes, Guy's Hospital Medical School. <sup>14</sup>C-Oestrogen was purchased from the Radiochemical Centre (4-<sup>14</sup>C-oestradiol, 10  $\mu\text{Ci}$  per 510 ml).

Rat albumin, and rat IgG from normal rat serum and immune serum were labelled with <sup>125</sup>I (specific activity 14  $\text{mCi mg}^{-1}$  iodine, Amersham) using the chloramine T method<sup>13</sup>. The extent of <sup>125</sup>I incorporation was assayed after precipitation of the labelled proteins—in the presence of carrier protein—with 10% trichloroacetic acid (TCA) and by the analysis of radioactivity in strips obtained from cellulose acetate electrophoretic patterns, as previously described<sup>14</sup>.

The labelled proteins were injected into the peritoneal cavity of newborn rats, on the first, third, fourth and seventh day after birth. The newborn animals—and the adult rats, used as controls—were killed between 3 and 24 h after the injections and blood was collected from the jugular vein. Samples of CSF, free of blood, were collected from the cisterna magna<sup>15</sup>.

In the 1-d-old rats about 20 and 10% of <sup>125</sup>I-albumin and <sup>125</sup>I-IgG, respectively present in blood, were detected in CSF 3 h after the injections into the peritoneal cavity. The labelled proteins could not be detected in CSF obtained from rats 7 d old or from adult rats, 3 h after the injections.

Figure 1 shows the results of one of the experiments in which <sup>125</sup>I-IgG isolated from normal rat serum or from rat immune serum containing antibodies against rat brain antigens, were used. Two newborn rats were injected intra-peritoneally with <sup>125</sup>I-IgG and two newborns with <sup>125</sup>I-IgG

Table 1 Levels of plasma proteins in foetal CSF

Foetal age (weeks)	Total proteins (mg ml <sup>-1</sup> )	Albumin (µg ml <sup>-1</sup> )	AFP (µg ml <sup>-1</sup> )	Transferrin (µg ml <sup>-1</sup> )	IgG (µg ml <sup>-1</sup> )	β2-Microglobulin (µg ml <sup>-1</sup> )	Causes of abortion
14	1.10	380	650	NT	25	NT	S
14	1.25	400	720	NT	NT	NT	S
16.5*	NT	NT	1,220	NT	NT	NT	S
18*	2.05	1,024	420	10	72	1.02	T
19	2.75	1,050	600	35	63	0.96	S
19.5*	NT	NT	1,040	NT	NT	NT	S
18-20	4.50	1,930	600	40	60	1.24	T
20	7.70	4,100	625	150	168	1.68	S
23.5	5.90	3,000	190	80	163	1.40	T
24	7.30	3,030	160	140	109	NT	T
25*	3.02	1,200	60	25	109	1.56	T
25*	3.95	840	71	NT	117	1.09	T
25*	3.20	680	52	10	60	0.66	T
40	2.5	NT	1	NT	71	NT	S
Normal adult CSF†	—	140-200	—	10-30	20-40	—	—

NT, not tested; S, spontaneous; T, therapeutic.

\* Levels of AFP already reported<sup>1</sup>.† From Laterre (ref. 2); mean levels (µg ml<sup>-1</sup>).

rat anti-rat brain. After 3 h the animals were killed and the radioactivity was measured in blood and CSF. Another four newborn rats from the same litter were injected when 4 d old and the remaining three at 7 d of age. About 10% of <sup>125</sup>I-IgG present in blood was detected in the CSF from 1-d-old rats. In the newborn rats injected with rat IgG antibodies against brain antigens the CSF contained over 20% of the labelled protein present in blood. Higher values of labelled IgG were detected in the CSF of rats 4 d old injected with rat antibodies than in rats injected with normal IgG. In rats 7 d old, less than 1% of the labelled protein present in blood was detected in the CSF of the animals killed 3 h after injection (Fig. 1).

More than 96% of the radioactivity present in serum and CSF was precipitated by TCA. When these samples were analysed by cellulose acetate electrophoresis, the peaks of radioactivity in serum and CSF were detected in the area corresponding to the IgG region in the electrophoretic patterns.

Preliminary studies have also indicated that an equilibrium between the levels of radioactive albumin or IgG in blood and CSF is reached within 24 h of the intraperitoneal injection into 1-d-old rats.

<sup>14</sup>C-Oestradiol, injected intraperitoneally in newborn rats, was detected in CSF 4 h after injection; the amount of labelled hormone found in CSF was between 40 and 60% of that detected in blood.

These studies and the results of preliminary investigations using heterologous antibodies against rat brain antigens (M.A. and S.H.) have thus confirmed that the permeability of the blood-CSF barrier is not fully developed during foetal and perinatal life in man and rats<sup>1,16</sup>.

It has long been recognised that the development of brain and unfolding of behaviour are sensitive to modifications by environmental factors<sup>17</sup>.

Experimental studies, reviewed by Balazs<sup>18</sup>, have shown that abnormal hormonal states during foetal life can result in permanent behavioural disorders, whereas, in adults, they usually induce changes that are reversible.

We suggest that, in abnormal maternal states, the permeability of the foetal blood-CSF barrier to hormones and antibodies against antigens of the nervous system may have an important role in the production of long lasting damage to the developing brain and to the unfolding of behaviour. Experimental work in progress should confirm or disprove this hypothesis.

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Table 2 Ratios of levels of proteins in CSF

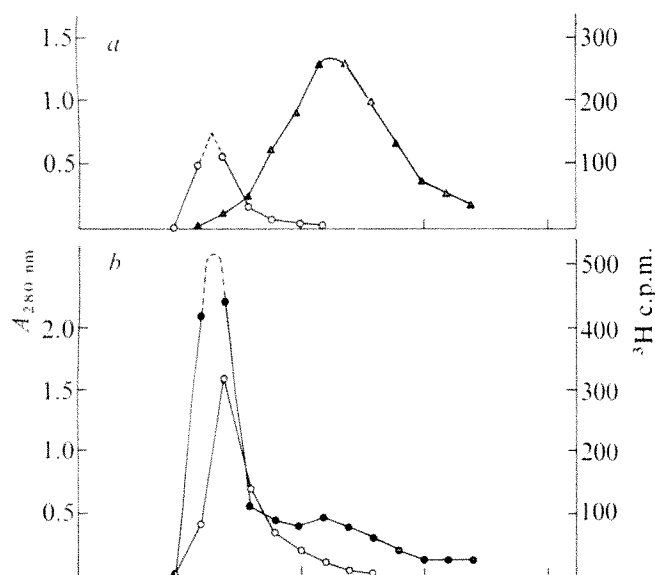
Foetus	Total protein/albumin	Alb./AFP	Alb./IgG	IgG/β2-microglobulin
1	2.89	0.58	15.2	—
2	3.12	0.55	—	—
3	—	—	—	—
4	2.00	2.43	14.22	70
5	2.61	1.75	16.66	65
6	—	—	—	—
7	2.33	3.21	32.16	48
8	1.87	6.65	24.40	100
9	1.96	15.78	18.40	116
10	2.40	18.13	27.79	—
11	2.51	20.00	11.09	69
12	4.70	11.83	7.11	107
13	4.70	13.07	11.33	90
14	—	—	—	—

## Antibodies to native tRNA in NZB/NZW mice

ANTIBODIES to native DNA, double-stranded RNA, and various synthetic polyribonucleotides occur with great frequency in patients with systemic lupus erythematosus (SLE)<sup>1</sup>. These antibodies are also present in the New Zealand mouse, particularly the NZB/NZW F<sub>1</sub> hybrid,

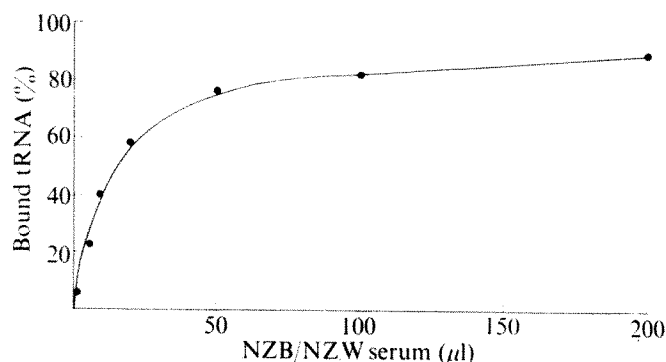
which is considered a good animal model for human SLE<sup>2</sup>. We report here the presence and characterisation of antibodies that bind tRNA, specifically in its native conformation, in sera from old NZB/NZW mice.

Figure 1 shows the results of gel chromatography experiments that demonstrate the presence of anti-tRNA antibodies in old NZB/NZW female mouse serum. The serum was treated to eliminate ribonuclease activity, incubated with radioactive *E. coli* tRNA, and then chromatographed on a small column of Sephadex G-100. Protein-bound tRNA was excluded from the gel while unbound tRNA was retarded (Fig. 1b). Control mice serum showed virtually no binding (Fig. 1a). We could also demonstrate tRNA binding activity in the NZB/NZW sera, but not in controls, using ammonium sulphate precipitation<sup>3</sup> or by precipitation of the complex with a rabbit anti-mouse antibody preparation.



**Fig. 1** NZB/NZW  $F_1$  hybrids and BALB/c control strains were obtained from colonies maintained at the National Institutes of Health. Mice were bled by orbital sinus puncture. About 20 sera were pooled, heated to 56 °C for 30 min and the immunoglobulin fraction was precipitated by  $(\text{NH}_4)_2\text{SO}_4$  at 50% saturation. This fraction was treated with charcoal to remove ribonuclease activity (manuscript in preparation) and then dissolved in phosphate-buffered saline to the volume of the original serum. Reaction mixtures contained 0.1 ml of serum, 0.15  $\mu\text{g}$  (2,000 c.p.m.) of *E. coli*  $^3\text{H}$ -tRNA (obtained by growing bacteria in medium containing  $^3\text{H}$ -uridine), and 0.01 M  $\text{MgCl}_2$  in a final volume of 0.12 ml. The mixture was incubated for 1 h at 4 °C and then applied to a  $0.7 \times 30$  cm Sephadex G-100 column. Chromatography was at 4 °C. Fractions of 0.65 ml were collected, measured for absorbance at 280 nm and counted in a scintillation counter. *a*, Incubation of  $^3\text{H}$ -tRNA with normal serum; *b*, incubation of  $^3\text{H}$ -tRNA with 8-month-old NZB/NZW female mouse serum.  $\circ$ ,  $A_{280}$ ;  $\bullet$ ,  $^3\text{H}$ -radioactivity.

Figure 2 shows the capability of the serum to bind essentially all added tRNA, indicating that the antibody recognises a general feature of all native tRNAs and not one or more of the rare nucleosides. (Alternatively, there may be subpopulations of antibodies which recognise each amino acid-specific tRNA.) Studies of antibodies made to conjugated yeast phenylalanine tRNA showed that they recognised only a modified nucleoside in the tRNA<sup>4</sup>. Figure 3 shows the age dependence of tRNA binding activity in the sera of female NZB/NZW mice. Each point represents the binding activity of sera pooled from 20 mice. The abrupt increase in binding at between 5 and 7 months suggests that these hybrid mice are programmed genetically



**Fig. 2** Binding curve of  $^3\text{H}$ -tRNA to 8-month-old NZB/NZW mouse female serum. Increasing amounts of serum were incubated with a fixed amount (75 ng) of  $^3\text{H}$ -tRNA. Binding capacity was measured by Sephadex chromatography as described in the legend to Fig. 1.

to produce anti-nucleic acid antibodies at a particular age. The production of anti-tRNA antibodies is very well correlated with other manifestations of the sickness<sup>2</sup>, such as renal immunoglobulin deposition, renal histological abnormalities, proteinuria and the production of anti-DNA antibodies<sup>5</sup>. The decline in tRNA binding activity after 10 months of age may result from the death of the most severely ill animals, decreased production or increased catabolism of the antibodies<sup>2</sup>.

NZB/NZW female mice (8–9 months old) sera were also assayed individually for tRNA and DNA-binding activity. All sera that bound DNA were also positive for tRNA. Five out of seven selected on the basis of very low double-stranded DNA binding (indistinguishable from normal mice), however, showed substantial binding for tRNA as well as for single-stranded DNA. These results suggest that anti-tRNA antibodies are more characteristic of old NZB/NZW mice than anti-native DNA antibodies.

Since the tRNA-binding protein in old NZB/NZW mouse serum is produced spontaneously in pathological conditions and not in response to a given immunogen, we tried to demonstrate that this protein is an immunoglobulin

**Table 1** Inhibition of  $^3\text{H}$ -tRNA and  $^{14}\text{C}$ -DNA-binding to NZB/NZW sera by nucleic acids and nucleic acid components

Inhibitor	Inhibition of $^3\text{H}$ -tRNA-antibody complex (%)	Inhibition of $^{14}\text{C}$ -DNA-antibody complex (%)
<i>E. coli</i> tRNA	63	25
Rat liver tRNA	63	
Denatured <i>E. coli</i> tRNA	25	34
Uridine	0	5
Cytidine	0	18
5'-Adenosine monophosphate	0	15
poly(A)	0	16
poly(rA)-poly(rU)	0	7
poly(rI)-poly(rC)	5	30
Double-stranded DNA	15	93
Single-stranded DNA	0	100
MS2 viral RNA	38	22
Q $\beta$ -viral RNA	38	8
Denatured MS2 viral RNA	13	20
16S ribosomal RNA	41	35
5S ribosomal RNA	43	26

$^3\text{H}$ -tRNA (0.15  $\mu\text{g}$ ) or native KB cell  $^{14}\text{C}$ -DNA<sup>6</sup> (0.05  $\mu\text{g}$ ) were mixed with 1.5  $\mu\text{g}$  of each of the specified inhibitors and NZB/NZW serum (100  $\mu\text{l}$  serum for the  $^3\text{H}$ -tRNA assay and 10  $\mu\text{l}$  serum for the  $^{14}\text{C}$ -DNA assay). Incubation was for 60 min at 4 °C. tRNA binding was analysed by Sephadex chromatography, as described in the legend to Fig. 1. DNA binding was measured by ammonium sulphate precipitation at 35% saturation. Denatured tRNA and denatured MS2 RNA were prepared by the method of Axelrod *et al.*<sup>13</sup>. MS2 RNA was given by Dr Rushizky, National Institutes of Health.

and not another binding protein or an enzyme. We used a multivalent rabbit anti-mouse  $\gamma$  globulin which had been purified by affinity chromatography (given by Dr R. Asofsky). Addition of 0.6 mg of this immunoglobulin to 50  $\mu$ l of the NZB/NZW serum, incubation at 4 °C for 5 d and centrifugation decreased the subsequent binding activity for  $^3$ H-tRNA by 96%. Furthermore, the purified anti-mouse  $\gamma$  globulin could precipitate all the tRNA bound to NZB/NZW serum in 2 h at 4 °C.

The specificity of old NZB/NZW mouse serum with respect to different nucleic acid antigens was tested by competition experiments (Table 1). The same antigens were also used to inhibit complex formation between anti-DNA antibodies and native  $^{14}$ C-DNA. The antibodies in NZB/NZW serum that bound tRNA and DNA behaved as distinct antibody populations, as the inhibition patterns of the two populations by different nucleic acids were very different (Table 1).  $^3$ H-tRNA binding was best inhibited by tRNA while  $^{14}$ C-DNA binding was best inhibited by double-stranded and single-stranded DNA.

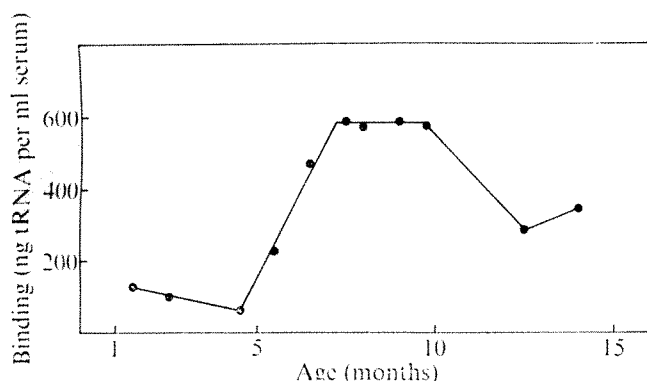


Fig. 3 About 20 female NZB/NZW mice of each age were bled and the sera were pooled, and assayed for tRNA-binding activity by Sephadex chromatography as described in Fig. 1.

Rat liver tRNA inhibited the tRNA-antibody reaction to the same extent as did *E. coli* tRNA. This supports the contention that the antibodies recognise a general feature in the structure of tRNA and not minor components which are somewhat different in the two species.

On the other hand, denatured tRNA which was fixed in its denatured conformation by formaldehyde, did not effectively compete with native tRNA, so this serum shows a marked specificity for the native conformation of tRNA. Nucleosides and nucleotides did not inhibit the binding reaction at all. The double-stranded synthetic polynucleotide poly(rI)-poly(rC) and DNA, which both bind very well to sera of NZB/NZW mice and SLE patients<sup>6</sup>, inhibited tRNA-binding to a very small extent. Conversely, single-stranded viral RNAs and ribosomal RNAs, which are believed to form hairpin secondary structures similar to those of tRNAs<sup>7,8</sup>, competed fairly effectively. In fact, 5S rRNA has been shown to compete with tRNA for a ribosomal binding site<sup>9</sup> and some viral RNAs can be aminoacylated by aminoacyl tRNA synthetases<sup>10</sup>. Denatured MS2 RNA cross linked with formaldehyde was a poor inhibitor compared with native MS2 RNA.

Attempts have been made in many laboratories to elicit antibodies specific for tRNA in experimental animals<sup>11,12</sup>. None of these antibodies has shown evidence of specificity for the secondary or tertiary conformation of the tRNA. Such antibodies would be useful for studies of the conformation and function of free and complexed tRNA in solution. The antibodies in the sera of the NZB/NZW mice may be useful in this connection and they should be

of value in understanding the molecular basis of lupus-like diseases.

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## Genetic control of type C virus of wild mice

THE genetic control of type C virus group-specific (GS) antigen expression<sup>1,2</sup>, infectious virus<sup>3-6</sup>, and tumorigenesis<sup>6,7</sup> has been described in crosses between strains of inbred laboratory mice with high and low incidence of leukaemia. We described a natural population of wild mice (*Mus musculus*) near Lake Casitas (LC) in southern California which showed a high level of indigenous type C virus activity in terms of GS antigen and infectious virus throughout their lifetime. When ageing was observed in the laboratory, LC wild mice showed a marked susceptibility to lymphoma<sup>8</sup>, epithelial tumours<sup>9</sup> and a neurogenic hind leg paralysis<sup>10</sup>. Virus transmission and *in vivo* neutralisation studies<sup>11</sup> established the indigenous type C virus as the critical determinant of the lymphoma and paralysis. To ultimately prevent these diseases by antiviral measures we have attempted to suppress LC virus expression by cross-breeding LC wild mice with a specific inbred laboratory mouse strain C57BL/10 Snell (designated B10). The B10 mouse was selected because of its homozygosity for two non-linked dominant alleles, *Fv-1<sup>b</sup>* and *MLv-1<sup>b</sup>*, the function of either of which might be expected to restrict virus expression in the hybrid progeny. Laboratory strains of mouse type C virus can be classified according to their *in vitro* host range as N-, B-, or X(xenotropic)-tropic depending on whether they grow preferentially in NIH Swiss or BALB/c embryo cells or replicate only in non-murine cells<sup>12,13</sup>. Field isolates from LC mice have an unusually wide *in vitro* host range ('amphotropic') but are N-tropic for mouse cells (S. Rasheed, V.K., and M.B.G., unpublished). B10 mice, within the first year of life, show a low incidence of both N- and B-tropic infectious virus<sup>14</sup> and also harbour an endogenous X-tropic genome<sup>15</sup>. The *Fv-1<sup>b</sup>* allele from the B10 parent should limit the replication of N-tropic virus within the LC×B10 progeny<sup>15</sup>, presumably by interfering with the integration or transcription of the proviral DNA<sup>16</sup>. The *MLv-1<sup>b</sup>* allele, the function of which is unknown, might restrict either N-, B-, or X-tropic virus expression in the progeny since this allele completely suppressed GS antigen expression in B10 crosses with the



congenic resistant (58N) strain<sup>17</sup>. Although the genetic mechanism has yet to be established, our findings indicate that control of type C virus expression can be accomplished by genetic means in wild mice.

Parental 6-month-old B10 mice (Jackson Laboratory) were reciprocally bred with newly trapped LC wild mice. Based on their weight of 10–15 g the LC parental mice were estimated to be 6 months old. A laboratory breeding colony of LC mice was also established. Parental mice were splenectomised before breeding and the segregating progeny from the various crosses (Table 1) were splenectomised at 4–6 weeks of age. Spleen extracts (10% w/v) were tested for GS (p30) antigen by the complement fixation (CF) test. Six male and five female LC mice whose spleens were positive for GS antigen were selected for the parental mating with separate B10s. All the parental and backcross B10 mice showed an absence of detectable spleen GS antigen.

The segregation of GS antigen expression in the different crosses is shown in Table 1. In striking contrast to the high prevalence and titre of detectable spleen CF GS antigen in the LC parental (72.6% GS positive, 49.5%  $\geq 1:4$ ), and 4–6-week-old LC mice born in the laboratory (66.7% GS positive, 40.0%  $\geq 1:4$ ), there was an almost complete absence of detectable spleen GS antigen in the 4–6-week-old reciprocal LC $\times$ B10 F<sub>1</sub> hybrids (1.4%) and in the reciprocal F<sub>1</sub> (2.3%) and F<sub>2</sub> (1.7%) B10 backcrosses. The negligible occurrence of detectable GS antigen at weanling age in the F<sub>1</sub> hybrids and B10 backcross progeny indicates a dominant negative effect on this form of viral expression.

To determine if suppression of GS antigen expression was temporary or long lasting, we tested 10% extracts for

**Table 1** Type C virus GS antigen expression in crosses between LC wild mice and B10 inbred mice

Generation of cross†	Spleen GS antigen (CF)*			
	1:2	%	$\geq 1:4$	%
Parental strains:				
B10	0/73‡	—	0/73	—
LC	207/289	72.6	143/289	49.5
F <sub>1</sub> generation:				
B10 $\times$ LC	1/111	1.4	0/111	—
LC $\times$ B10	1/37	—	0/37	—
F <sub>2</sub> generation:				
(B10 $\times$ LC)F <sub>1</sub> $\times$ (LC $\times$ B10)F <sub>1</sub>	4/110	3.7	1/110	—
(LC $\times$ B10)F <sub>1</sub> $\times$ (B10 $\times$ LC)F <sub>1</sub>	7/106	6.6	1/106	—
(B10 $\times$ LC)F <sub>1</sub> $\times$ (B10 $\times$ LC)F <sub>1</sub>	11/115	9.6	1/115	—
(LC $\times$ B10)F <sub>1</sub> $\times$ (LC $\times$ B10)F <sub>1</sub>	27/153	17.6	11/153	—
F <sub>1</sub> backcross to B10:				
B10 $\times$ (B10 $\times$ LC)F <sub>1</sub>	0/62	—	0/62	—
B10 $\times$ (LC $\times$ B10)F <sub>1</sub>	1/36	2.3	1/36	—
(B10 $\times$ LC)F <sub>1</sub> $\times$ B10	1/67	—	0/67	—
(LC $\times$ B10)F <sub>1</sub> $\times$ B10	3/56	—	2/56	—
F <sub>2</sub> backcross to B10:				
B10 $\times$ (B10 $\times$ LC)F <sub>2</sub>	1/29	—	0/29	—
B10 $\times$ (LC $\times$ B10)F <sub>2</sub>	0/9	1.7	0/9	—
(B10 $\times$ LC)F <sub>2</sub> $\times$ B10	—	—	—	—
(LC $\times$ B10)F <sub>2</sub> $\times$ B10	0/22	—	0/22	—
F <sub>1</sub> backcross to LC:				
LC $\times$ (B10 $\times$ LC)F <sub>1</sub>	12/33	36.4	3/33	—
LC $\times$ (LC $\times$ B10)F <sub>1</sub>	17/36	47.2	7/36	—
(B10 $\times$ LC)F <sub>1</sub> $\times$ LC	4/91	4.4	1/91	—
(LC $\times$ B10)F <sub>1</sub> $\times$ LC	17/51	33.3	10/51	—
F <sub>1</sub> laboratory bred LC:				
LC $\times$ LC	50/75	66.7	30/75	40.0

\* Spleens were removed from parental LC and B10 mice in young adulthood (2–6 months of age) and from progeny mice at 4–6 weeks of age. 10% extracts were tested by CF at 1:2 and 1:4 antigen dilutions with a 1:2 dilution of rat sera pools (MVS 30, 3490, 4918) prepared in Fischer rats bearing Moloney sarcoma virus tumour transplants. Specificity of sera confirmed by comparison with guinea pig antisera to electrofocus purified MuLV (P30) antigen<sup>22</sup>.

† Terminology used in these crosses designates female on left and male on right of  $\times$ .

‡ Number positive/number tested.

**Table 2** Type C virus isolation from wild-inbred parental and hybrid mice

Generation or cross	Spleen CF GS positive		Spleen CF GS negative	
	Spleen	Tail	Spleen	Tail
B10 parental	—	—	1/10	1/5
LC parental	4/4	3/5	—	—
F <sub>1</sub>	1/2	0/2	0/10	1/10
F <sub>2</sub>	1/13	0/10	0/24	0/10
F <sub>1</sub> backcross B10	1/1	0/5	0/9	0/5
F <sub>1</sub> backcross LC	9/9	4/6	0/9	0/2

For virus isolation 0.1 ml 10% spleen extract (stored at  $-70^{\circ}\text{C}$  for several months) and 0.1 ml fresh 20% tail homogenates (not necessarily from the same individual mice) were assayed undiluted for induction of CF GS antigen after 21 d (COMUL test) on wild mouse SC-1 (ref. 20) and rabbit SIRC<sup>21</sup> cells. Antisera used were as in Table 1.

CF GS antigen from a random sample of healthy F<sub>1</sub> progeny and F<sub>1</sub> backcrosses to B10 progeny after various periods of observation. We have shown that in 3-week-old LC mice, the degree of type C virus expression in the liver is equal to that in the spleen (M.B.G., V.K. and R.J.H., unpublished). GS antigen was detected in none of 23 F<sub>1</sub> hybrids and 93 F<sub>1</sub> backcross to B10 progeny observed for an average of 7 months (range 3–10 months). The repression of GS antigen thus seemed fairly long lasting.

In the F<sub>2</sub> reciprocal crosses, spleen GS antigen was found in an average of 10.0% (range 3.7–17.6%), mostly in low titre (1:2), (Table 1). Although these results suggested a two gene suppressive effect on GS antigen expression which would have predicted 7% GS antigen positive in the F<sub>2</sub> generation, we could not confirm this hypothesis by analysis of the GS antigen ratios in the F<sub>1</sub> backcross to LC mice. A greater prevalence of spleen GS antigen was observed in backcross matings with LC females (average 42.0%) compared with LC males (average 14.8%), (Table 1). This finding suggested either a positive maternal effect on virus expression by epigenetic spread of virus to offspring from the heavily infected LC maternal reproductive tract (M.B.G., V.K. and R.J.H., unpublished) and milk<sup>18</sup> or a negative maternal effect, presumably from neutralising antibodies passed to their offspring by the virus-antigen-stimulated hybrid mothers<sup>4</sup>. In the latter instance the only segregation data that would be directly interpretable are those backcross matings with LC mothers. Viewed in this way our results are most compatible with segregation of a single gene, that is, 42% observed compared with 50% predicated GS-positive progeny in the LC maternal backcross. The single gene probably responsible for this suppression of GS antigen would be the *Fv-1* rather than *MLv-1<sup>b</sup>* since the *Fv-1* locus has been shown to be the major determinant of virus expression and spontaneous leukaemia in hybrids with AKR and other high-leukaemia *Fv-1<sup>a</sup>* laboratory mouse strains<sup>4,6</sup>. A more extensive genetic analysis will be required, however, to rigorously establish the gene locus primarily responsible for this dominant control of virus expression in LC wild mice.

A sample of the 10% spleen extracts collected at 4–6 weeks of age and previously tested for GS antigen by CF, and fresh 10% tail clip homogenates collected at 6–12 months of age were assayed for infectious virus by the COMUL test<sup>19</sup> on mouse (SC-1) and rabbit (SIRC) cells (Table 2). SC-1 cells (Dr J. Hartley, NIAID, NIH) are a cloned wild mouse embryo cell line which seem to lack both the *Fv-1<sup>a</sup>* and *Fv-1<sup>b</sup>* alleles and are thus equally susceptible to N- and B-tropic viruses<sup>20</sup>. SIRC cells are a rabbit cell line<sup>21</sup> susceptible to the X-tropic viruses of laboratory mice and the amphotropic viruses from LC wild mice (S. Rasheed, V.K., and M.B.G., unpublished). Virus was recovered in both SC-1 and SIRC cells from all the high titred ( $\geq 1:4$ ) GS-positive spleens (13 of 13) and from most (7 of 11) of the tail homogenates tested from the parental and backcross LC mice. By contrast,

virus was recovered in only SC-1 cells from just 6 of 116 total assays from spleen and tail of parental B10 and F<sub>1</sub>, F<sub>2</sub>, and B10 backcross progeny, including a sample from those few mice in these groups whose spleen extracts were weakly positive (1:2) for CF GS antigen. Thus, infectious amphotropic virus could readily be recovered from spleen or tail only of parental and backcross LC mice which had high titred spleen GS antigen. In no instance was virus isolated on SIRC and not on SC-1 cells. Therefore, it is unlikely that any of the other isolates represented the X-tropic class of endogenous type C virus. Suppression of GS antigen expression in LC × B10 hybrids was clearly correlated with a suppression of infectious type C virus production.

Observation of (LC × B10) F<sub>1</sub>, F<sub>2</sub> and backcross progeny into old age is now under way to determine if resistance to lymphoma, epithelial tumours, and paralysis will occur in those hybrids segregating for lack of GS antigen and infectious virus expression.

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## Second genetic locus in the HLA region for human B-cell alloantigens

THE ability to separate human peripheral blood T and B lymphocytes has allowed serological identification of specific B cell antigens, and several lines of evidence indicate that these antigens are controlled by a gene or genes of the HLA genetic region<sup>1</sup>. Van Rood *et al.* presented evidence for an antigen which seems to be associated with, or closely linked to, the

locus responsible for mixed lymphocyte stimulation (HLA-D)<sup>2</sup>. Our results in family studies suggest that there are a number of specific B-cell alloantigens that are controlled by genes linked closely to genes controlling the expression of HLA alloantigens<sup>3,4</sup>.

B-cell alloantigens have been defined in murine systems and have been designated Ia antigens, which seem to be controlled by genes mapping within the H-2 complex<sup>5</sup>. At least three genes have now been identified controlling the different Ia antigens<sup>6</sup>. If the genetic region controlling cell-surface antigens on lymphocytes is analogous in the murine and human species, it seems probable that several genes might control the expression of human B-cell alloantigens. The results, presented here, of studies on B-lymphocyte antigens in a family where one member was an HLA recombinant provides evidence that such is the case.

We used human sera containing antibodies detecting B-cell specific antigens, obtained from multiparous women of a religious sect, the Amish, who, for cultural and religious reasons, live in rather isolated communities and, as a result, marry exclusively within the community. Peripheral blood lymphocytes were obtained from the father, mother and six children from the Amish family under study.

T and B lymphocytes were separated as previously described<sup>4</sup> and tested by cytotoxicity with 34 antisera that were known to detect specific B-cell antigens and which did not react with HLA alloantigens. Briefly, this technique for separation and typing is as follows. Peripheral blood lymphocytes were isolated from heparinised whole peripheral blood by sedimentation with Ficoll-Hypaque, macrophages were depleted with carbonyl iron, and  $2 \times 10^5$  lymphocytes were dispensed into microtitre plates that had been coated with antigen-antibody complexes. The lymphocytes were allowed to settle, resulting in adherence of the cell population bearing Fc receptors. The plates were then flooded, inverted, and the non-adherent cells recovered, concentrated, and  $10^3$  cells dispensed into microtitre plates for testing against the same sera that were used to test the adherent cell population. The adherent and the non-adherent cells were tested in triplicate with each of the 34 antisera and appropriate controls consisting of normal human sera and a known lymphocytotoxic sera. Whole rabbit serum was used as a source of complement.

Antiserum (5 µl) was allowed to incubate with the cells for 1 h, the cells were washed with balanced salt solution containing 10% heat-inactivated foetal bovine serum and 5 µl of complement added. After incubation the complement was removed, the cells washed, and 5 µl of complement added for an additional 1-h incubation period. Complement was then removed and Trypan blue added. The reaction was deemed cytotoxic positive when > 30% of the cells (over that seen in the negative controls) incorporated Trypan blue. The background in negative controls was within the range 0–10% cells stained.

The results of the B-lymphocyte typing in this family are shown in Table 1 together with the HLA genotype of each family member. The serum reactivity reported here showed exclusive reactivity with B cells and no activity against the non-adherent or T-cell population. All the antisera can be assigned to HLA haplotypes and seven can be assigned to genetic subregions within the haplotype. Serum 640 was the unique marker for the maternal 1–8 haplotype inherited by sib 5. Sera 289 and 43 reacted against markers for the maternal 3–7 haplotype inherited by sibs 1, 2, 3, 4 and 6. Sera 35, 76, and 177 defined the 1–12 paternal haplotype inherited by sibs 3 and 4 and sera 590, 67, 322, 251 and 370 were directed against the 9–27 haplotype inherited by sibs 1, 2 and 5. Sib 6 was known from previous HLA typing to be a recombinant between the HLA-A and HLA-B loci. His cells were reacted against by sera 289 and 43, and by 35, 76, 322, 251 and 370. The reactions of 289 and 43 are clearly against the 3–7 haplotype. Reactions of sera 35 and 76 are shared with those sibs possessing HLA-A2 and are thus directed against an HLA-A region product. Sera 322, 251

**Table 1** B lymphocyte typing in HLA recombinant family

	Parents				Children			
	Mother		Father		1. Edn 2. Erm		3. Erv 4. Ida	
HLA type	1-8	3-7	2-12	9-27	3-7	9-27	3-7	2-12
Serum reactivity	640	289	35	590	289	590	289	35
with assignment	—	43	76	67	43	67	43	76
to HLA haplotype	—	—	177	322	—	322	—	177
				251		251		251
				370		370		370

	Children				6. Rom (Recombinant)			
	1. Edn 2. Erm		3. Erv 4. Ida		5. Nel		6. Rom (Recombinant)	
HLA type	3-7	9-27	3-7	2-12	1-8	9-27	3-7	2-27
Serum reactivity	289	590	289	35	640	590	289	35
with assignment	43	67	43	76	—	67	43	76
to HLA haplotype	—	322	—	177	—	322	—	322
	—	251	—	—	—	251	—	251
	—	370	—	—	—	370	—	370

B lymphocyte typing in a family where one offspring was an HLA recombinant. Serological reactions with B cells are assigned to a particular HLA haplotype as reactions occurred in parents and offspring. The different sera reacting with these cells have been arbitrarily assigned numbers such as 640, 289, 43, and so on.

**Table 2** HLA haplotypes\* of three families and the reactions of B-cell antisera

HLA haplotype	1-8	2-7	2-7	29-BW40	2-7	1-BW35	9-BW15	1-8	3-7	2-12	9-27
B-cell sera reacting	1	52	176	35	52	9	107	640	43	35	67
	35	289	177	52	289	76	177		289	76	251
	43		590	192		107	189			177	322
	76			244		196	196				370
	116			289		359					590
	124			590							
	189										
	207										
	386										

\*Three large families were tested for B lymphocyte antigens. Listed above are the HLA haplotypes found in these families and the reactions of their cells with the B-cell typing sera.

and 370 react with the HLA-B region product as shown by reactions of sibs 1, 2, 5 and 6. Note that sib 6 is not reactive with sera 590 and 67 associated with the intact 2-12 haplotype. This failure to react is not an artefact, as shown by absorption studies. Sera 177, 590 and 67 were absorbed with cells from sib 6 and retested against cells from the father. All remained fully active, showing that the antigens were indeed absent from the recombinant.

This shows clearly that the B-cell antigens are coded for by at least two genetic loci, one associated with the HLA-A portion of the haplotype and one with the HLA-B portion. This family study is not informative as to the precise location of the genes within the HLA-A and HLA-B regions. We can, however, be fairly sure that the genes for B-cell antigens are separate from the determinants for HLA-A and HLA-B from three lines of evidence. First, the sera react only against B cells; their failure to react against T cells argues against their being antibodies to the A, B, or C loci of HLA. Second, serum 590 was produced in a woman HLA 3-7/28-14 against a husband 3-7/3-BW55, so cannot react with HLA-A9, and a similar argument can be made for antiserum 67. The serum donor was 2-BW35/28-27 and her husband 2-7/11-27. Third, when tested on a random panel of B cells from subjects characterised for HLA, the antisera did not correlate with the distribution of HLA specificities. Further, when the reactions of the sera were examined in other families, different patterns of reactivity were observed. For example, in one family 590 reacted together with sera 177 and 176 to define the 2-7 haplotype in a group of nine sibs and in another family serum 177 reacted with four sibs inheriting the 9-BW15 haplotype whereas 590 reacted

with five sibs inheriting the 29-BW40 haplotype (Table 2). Thus not only do the different anti-B-cell sera sort with different HLA haplotypes in different families, they also seem to detect different B-cell specificities.

The present evidence indicates that there is a minimum of two loci for B-cell antigens within the HLA region. From the reactions of HLA haplotype-associated B-cell antisera in different families it seems probable that this is a minimum estimate and that the number of genetic loci may be considerably higher.

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## Electron transfer across membranes using vitamin K<sub>1</sub> and coenzyme Q<sub>10</sub> as carrier molecules

CONTINUOUS redox processes have been effected between aqueous reactants, separated by liquid membranes in which coenzyme Q, or vitamin K, is present as a carrier molecule. These membranes show chemical specificity and function which relate closely to certain biological electron transfer processes.

The neutral lipid, coenzyme Q, is an essential link in the electron-transfer chain in mitochondria, and vitamin K may have a similar function in chloroplasts and certain bacteria.

We investigated the conditions in which these amphipathic quinone lipids may act as electron transfer agents, using solutions of vitamin K<sub>1</sub> and coenzyme Q<sub>10</sub> in hydrocarbon solvents as liquid membranes. These bulk membranes, being less dense than water, separated aqueous reducing agents and potentially reducible substrates (Fig. 1). The membranes may be considered to be composed of three parts; two interfacial monolayers, in which hydrophilic quinone or quinol groups are present at the aqueous interfaces, and the bulk hydrocarbon solution phase. Since there is a dynamic equilibrium between the monolayers and the bulk membrane phase, molecules reduced on one side may be transferred by diffusion to the opposite interface where they may reduce the aqueous substrate (A in Fig. 1). When both reductant and substrate are insoluble in the membrane phase each quinol (reduced) molecule may act as a carrier for two electrons and two protons. Electro-neutrality is thus maintained in both aqueous phases and a continuous process of electron transport may be maintained.

The normal reduction potentials ( $E'_0$ ) for both vitamin K<sub>1</sub> and coenzyme Q<sub>10</sub> at pH 7 are close to 0 V (ref. 1) and so they are obtained in their oxidised forms in atmospheric conditions. To establish the requirements for a biphasic reduction, hexane solutions of quinone were equilibrated in oxygen-free conditions with various common aqueous reducing agents which had been shown to have no solubility in the hexane phase. Vitamin K<sub>1</sub> was reduced by only two reducing agents, methyl viologen and reduced flavin mononucleotide, FMNH<sub>2</sub>, (a prosthetic group of flavoproteins) both in phosphate buffer at pH 7. Coenzyme Q<sub>10</sub> was less specific and also was completely reduced by aqueous dithionite and borohydride ions. For vitamin K<sub>1</sub>, particularly, it seems that one-electron transfer agents are required. In support, electron spin resonance (ESR) spectra taken at the aqueous-liquid membrane interface during reduction with methyl viologen showed the presence of semi-quinone radicals as transient species for both quinones.

Of particular relevance to the role of coenzyme Q in natural membranes were the observations that, although aqueous solutions of NADH and NADPH were unable to reduce the

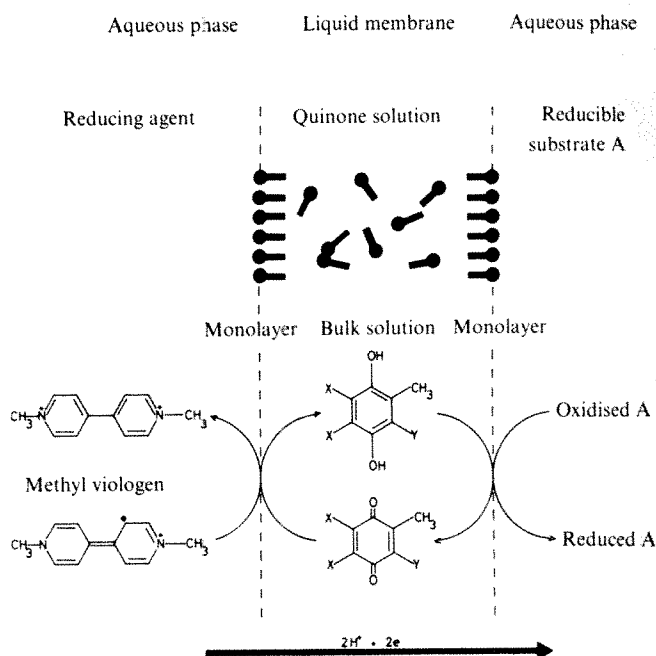


Fig. 1 Diagrammatic representation of the aqueous membrane model.

quinone directly, they may do so indirectly by a stepwise reduction involving FMN as an essential intermediate. These compounds, with their associated enzymes, have been shown to act in this sequence in the respiratory chain. Our results therefore indicate that limiting kinetic factors intrinsic to the electron-transfer process within the mitochondrion are already present in this simple analogue.

To establish continuous electron-transfer processes, experiments were conducted in an H cell, in which the quinone-hexane solution formed a liquid bridge between the two aqueous phases in the lower limbs of the cell. For convenience, reduced methyl viologen in phosphate buffer (pH 7) was used as reductant and various substrates were tested for reactivity. The apparatus was flushed with nitrogen and sealed. Equilibrations were conducted at 25 °C and the cell was vibrated gently to facilitate mixing in each of the three phases. Results, summarised in Table 1, show that a number of common redox indicators, used in biological studies on the respiratory chain, are completely reduced. Coenzyme Q<sub>10</sub> reduced ferric ion in acidic conditions and also iron III *o*-phenanthroline complex, which was reduced to ferroin. That cytochrome *c* was also completely reduced, indicated that these liquid membranes may be a suitable model for certain steps in the respiratory chain. Protonated molecules, such as reduced methylene blue, or protonated, oxidised, DCIP (the latter present to only 5% at pH 7) are soluble in hexane and are transported across the membrane. In mitochondria, this would provide a mechanism

Table 1 Tests of the reducing abilities of reduced vitamin K<sub>1</sub> and coenzyme Q<sub>10</sub> with a variety of common substrates

Substrate	$E'_0$ (ref. 1) (V)	$E_0$ (ref. 2) (V)	Reduced vitamin K	Reduced coenzyme Q	Comments
2,6-Dichlorophenol-indophenol	0.217		+	+	Back transfer of protonated (red) form
Indophenol	0.228		+		
Methylene blue	0.011		+	slight	Back transfer of reduced (leuko) form
Thionine	0.063		+	—	
FeCl <sub>3</sub>		0.77		+	
(Fe( <i>o</i> -phen) <sub>3</sub> )Cl <sub>3</sub>	1.12		+	+	Back transfer of uncomplexed <i>o</i> -phenanthroline
Cytochrome <i>c</i>	0.26		+	+	

The pH was maintained at 7 for each aqueous substrate except ferric chloride (which was in a solution of 0.1 M HCl) and the concentrations of the test solutions ranged from 0.01–0.10 mM.



of selective transport of molecules made lipid soluble by reduction or protonation.

Preliminary results have been obtained for the kinetics of reduction of aqueous substrates. The same H-cell format was used with the lower limbs (containing aqueous phases) replaced by optical cells. These aqueous limbs were stirred magnetically and turbulence and mixing of the quinone membrane solution obtained by gently rocking the whole apparatus. Tests showed that the rate of reduction of substrate was independent of whether or not the quinone was fully reduced at the start of the experiment. This confirmed that reduction by methyl viologen was rapid and that adequate agitation of bulk membrane had been obtained. Rates of transfer were insensitive to tenfold reduction in quinone concentration in the membrane, indicating a saturation effect typical of carrier-mediated transport. The reduction kinetics of methylene blue were first order with respect to the oxidised form and varied with stirring. These results are compatible with a diffusion-controlled mechanism, dependent only on the rate of diffusion of oxidised substrate from the bulk aqueous phase to the membrane interface. Electron transfer may thus be very rapid in this case.

Bimolecular lipid membranes have not been obtained, but stable thicker membranes  $\sim 1 \times 10^2$ – $3 \times 10^2$  nm have been formed in Teflon orifices using decane solutions of both quinones. These have been shown to function in the same way as the bulk membranes described above.

Apart from the obvious biological implications, such membrane systems provide a new sensitive method for the preparation and characterisation of solutions of reduced or oxygen-sensitive compounds, without the added complication of chemical contamination (other than protons required to ensure electroneutrality). In particular, solutions of pure reduced cytochrome *c* and of the very oxygen-sensitive reduced vitamin  $K_1$  in hexane were obtained in this study. The latter, previously uncharacterised, exhibited an intense maximum at 245 nm ( $\epsilon = 44,500$  l mol<sup>-1</sup> cm<sup>-1</sup>) and a broader weaker band at 325 nm.

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## Effect of experimental diabetes and insulin on phosphorylation of rat liver ribosomal protein S6

ONLY one rat liver ribosomal protein (S6) is phosphorylated *in vivo*<sup>1</sup>. During hepatic regeneration the phosphorylation of S6 is increased by an order of magnitude, and derivatives are generated (in some experiments as many as five) which contain increasing numbers of phosphoserine residues<sup>1</sup>. Very similar changes in the phosphorylation of S6 are caused by administration to animals of glucagon or cyclic AMP (A.M.G. and I.G.W., unpublished). Indeed, a number of hormones including adrenocorticotrophic hormone (ACTH)<sup>2</sup> and thyroid hormone<sup>3</sup> increase the phosphorylation of ribosomal protein, although in the latter instances, the identity of the phosphoprotein has not been established. These observations have led us to consider the possibility that cyclic AMP is the mediator of the phosphorylation of

S6 (ref. 4). The concentration of cyclic AMP in the liver is increased in diabetic animals and restored to normal by insulin<sup>5</sup>, although it is still moot whether the effect of the hormone on the level of the cyclic nucleotide accounts for all (or even any) of its actions on hepatic cells<sup>6</sup>. We have tested the effect of experimental diabetes and of insulin administration on the phosphorylation of rat liver ribosomal protein: the phosphorylation of S6 was markedly increased by diabetes, and reduced towards the normal level by insulin administration.

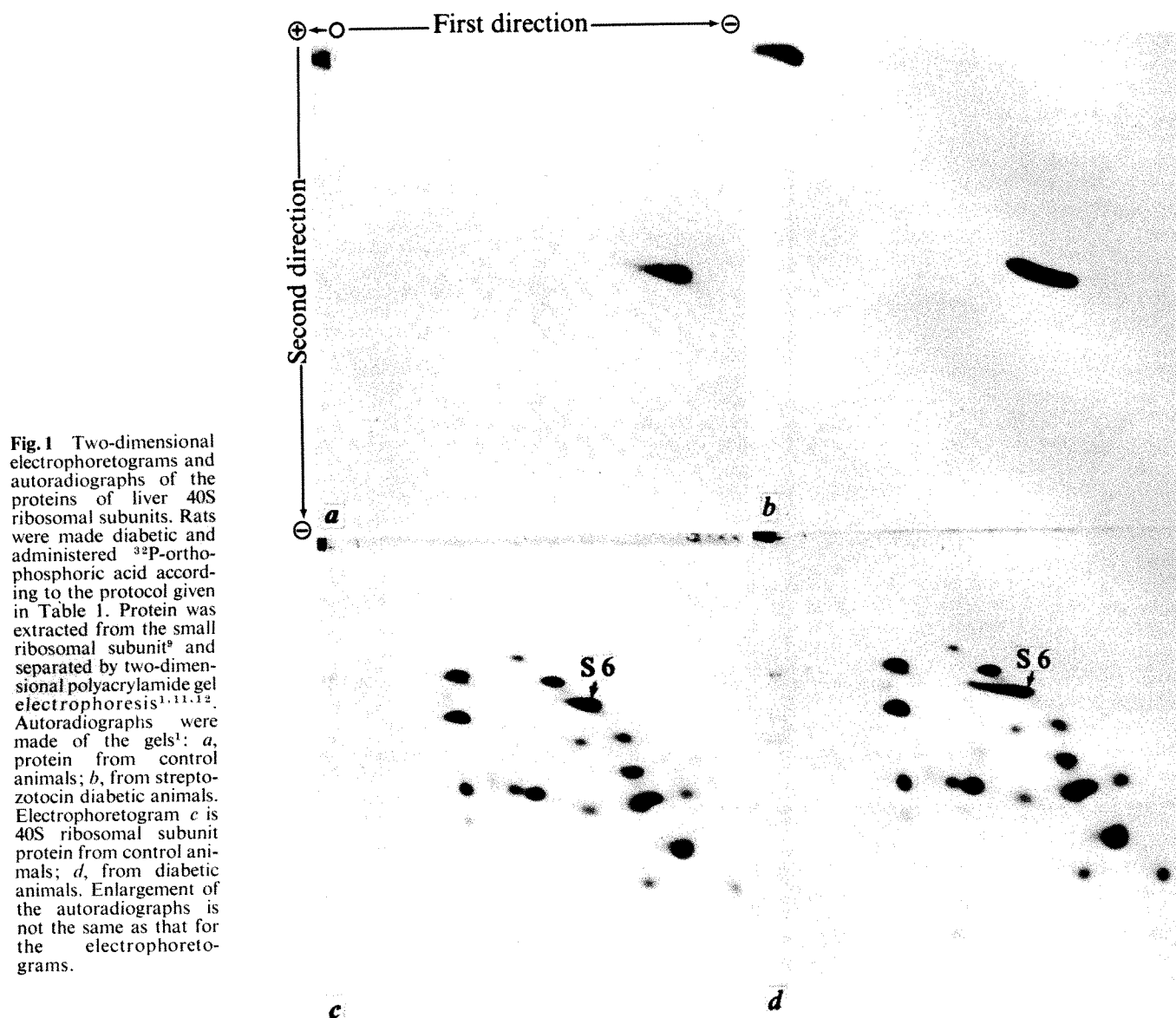
Diabetes caused a 3.5-fold increase in the <sup>32</sup>P-radioactivity associated with liver 80S ribosomes (Table 1); the administration of insulin (5 units per 100 g body weight) to diabetic animals reduced the radioactivity associated with the particles by about one-half in 90 min; phosphorylation, however, was not restored to normal during that period (Table 1). Not all of the radioactivity associated with liver ribosomes after administration of <sup>32</sup>P-orthophosphoric acid to rats is in ribosomal protein<sup>1</sup>. To test whether diabetes and insulin had actually affected the phosphorylation of ribosomal protein, subparticles were prepared from the ribosomes and the protein was extracted and the specific radioactivity determined. Diabetes increased the phosphorylation of the protein of the small ribosomal subunit by more than 3.5 times (Table 1); insulin administration decreased the specific <sup>32</sup>P-radioactivity of the protein of the 40S subparticle by somewhat less than one-half (Table 1). The effect of diabetes and insulin on phosphorylation of the small subunit protein can account for the alteration in radioactivity associated with 80S ribosomes. Very little radioactivity was in the protein of the large subunit and it was not appreciably changed by diabetes or insulin (Table 1). We have found<sup>1</sup> that phosphorylation of rat liver ribosomal protein *in vivo* is entirely in the 40S subunit; the radioactivity apparently associated with the large subunit is actually due to contamination with a small amount of 40S subparticles<sup>1</sup>.

All of the radioactive phosphate incorporated into rat liver ribosomes *in vivo* is found in S6 (ref. 1). It seemed likely, therefore, that it was the phosphorylation of S6 that was affected by diabetes and insulin. To test the possibility the proteins of the 40S subunit were separated by two-dimensional polyacrylamide gel electrophoresis and radioautographs made of the gels (Fig. 1). 40S ribosomal subunits from animals that had received <sup>32</sup>P-orthophosphoric

**Table 1** Incorporation of radioactivity from <sup>32</sup>P-orthophosphoric acid into rat liver ribosomes and ribosomal subunits

Source of liver ribosomes	<sup>32</sup> P-radioactivity		
	80S (c.p.m. per $A_{260}$ )	40S (c.p.m. per $\mu$ g protein)	60S (c.p.m. per $\mu$ g protein)
Normal	850	1.7	0.9
Diabetic	3,120	6.5	1.1
Insulin-treated diabetic	1,620	3.6	1.0

Male Holtzman rats (140–160 g) were starved for 24 h and injected intravenously with 65 mg per kg body weight of streptozotocin (lot no. 10518-GGS-37; provided by Dr W. E. Dulin, Upjohn) freshly dissolved in citrate buffer (pH 4.4). Control rats received the same volume of citrate buffer alone. Three days (68 h) after treatment with streptozotocin the fasting blood glucose levels were higher than 250 mg 100 ml<sup>-1</sup>. The diabetic animals were at that time divided into two groups of two or three rats each: one group received intraperitoneally 5 U crystalline porcine insulin (lot no. 615-063-10, containing 0.002% glucagon; provided by Dr O. Behrens, Eli Lilly) per 100 g body weight in saline. Control animals received a similar volume of saline. Animals (control, diabetic and insulin-treated diabetic) were injected intraperitoneally with 3 mCi <sup>32</sup>P-orthophosphoric acid (carrier-free, in 0.02 N HCl, 285 Ci mg<sup>-1</sup>, from ICN). Radioactive isotope was administered 90 min after animals received insulin or saline. All of the animals were decapitated 20 min after administration of the isotope. Livers were excised and ribosomes<sup>7</sup> and ribosomal subunits<sup>8</sup> prepared. Radioactivity in 80S ribosomal particles or in protein extracted from ribosomal subunits<sup>9</sup> was determined as described previously<sup>1</sup>. Concentration of protein in a sample was assessed<sup>10</sup> using bovine serum albumin as a standard.



**Fig. 1** Two-dimensional electrophoretograms and autoradiographs of the proteins of liver 40S ribosomal subunits. Rats were made diabetic and administered  $^{32}\text{P}$ -orthophosphoric acid according to the protocol given in Table 1. Protein was extracted from the small ribosomal subunit<sup>9</sup> and separated by two-dimensional polyacrylamide gel electrophoresis<sup>11,12</sup>. Autoradiographs were made of the gels: *a*, protein from control animals; *b*, from streptozotocin diabetic animals. Electrophoretogram *c* is 40S ribosomal subunit protein from control animals; *d*, from diabetic animals. Enlargement of the autoradiographs is not the same as that for the electrophoretograms.

acid, but no other treatment, had a single radioactive phosphoprotein (Fig. 1*a*). Comparison of the autoradiograph (Fig. 1*a*) and the stained two-dimensional gel (Fig. 1*c*) established that the protein was S6. Diabetes seemed to increase the specific radioactivity of S6 (Fig. 1*b*), although one could not be sure of that from the autoradiograph alone.

It was certain, however, that diabetes had changed the conformation of the protein. The zone containing S6 on the stained two-dimensional gel was elongated with a tail extending towards the anode (Fig. 1*d*); several (perhaps, as many as four) distinct derivatives of S6 could be discerned, each more negatively charged. Similar changes have been shown before<sup>1</sup> to be the result of phosphorylation of increased numbers of serine residues in the protein. The autoradiograph (Fig. 1*b*) conformed to that interpretation; the intensity of the zone described a gradient of specific radioactivity that was greatest in the anodal portion and least where one would expect the normal S6. Similar changes in S6 occur during hepatic regeneration<sup>1</sup>, as a result of the inhibition of protein synthesis with puromycin or cycloheximide<sup>4</sup>, and after administration of glucagon or cyclic AMP (A.M.G. and I.G.W., unpublished).

The increase in phosphorylation of S6 caused by diabetes cannot have been due solely to a change in the specific radioactivity of the intracellular pool of inorganic phos-

phate, or of ATP, as a result of a change in the transport of phosphate into hepatic cells. First, diabetes does not change the concentration of ATP in the liver<sup>12</sup>. But more important, one can see the highly phosphorylated derivatives of S6 on stained gels after two-dimensional electrophoresis of ribosomal protein from diabetic animals; they were not seen when ribosomal protein from normal animals was analysed. The demonstration of the derivatives of S6 on gel plates does not depend on changes in specific radioactivity and thus authenticates the enhancement of ribosomal protein phosphorylation as a result of diabetes.

Whether the change in S6, caused by insulin deficiency, is the result of increased phosphorylation or decreased dephosphorylation is not known. That cyclic AMP causes similar changes (A.M.G. and I.G.W., unpublished), and that the hepatic concentration of the cyclic nucleotide is elevated in diabetes<sup>3</sup>, suggests that it is increased phosphorylation resulting from the activation of a protein kinase that is responsible. The changes in S6 are by no means specific for diabetes: similar alterations occur during hepatic regeneration<sup>1</sup> and after administration of glucagon or cyclic AMP (A.M.G. and I.G.W., unpublished); and probably after administration of other hormones as well<sup>2,3,14</sup>. Insulin is the first agent, however, found to decrease phosphorylation of liver ribosomal protein. Insulin has also been reported to promote phosphorylation of

membrane and ribosomal protein in mammary gland stem cells<sup>15</sup>, although the phosphorylation of the ribosomes was not adequately characterised<sup>1</sup>.

The functional significance of the phosphorylation of S6 is not known, although the possibilities have been discussed<sup>1</sup>. Since the impact, if any, of the phosphorylation of S6 is not known, it is impossible to say whether the change bears a causal relation to the alterations induced by diabetes in protein synthesis<sup>16-18</sup>.

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## Phosphorylation of ribosomal proteins in HeLa cells infected with vaccinia virus

MODIFICATION of ribosomal proteins has been suggested as a mechanism controlling protein biosynthesis<sup>1</sup>. *In vivo* and *in vitro* phosphorylation of eukaryotic ribosomal proteins has been described and its possible functional relevance discussed<sup>2-5</sup>. Two-dimensional polyacrylamide gel electrophoresis has shown that only one, small ribosomal subunit protein, S6, is phosphorylated in rat liver<sup>6</sup>. The number of phosphoserine residues in this protein increases by an order of magnitude during liver regeneration<sup>6</sup> and also after administration of inhibitors of protein synthesis<sup>7</sup>. In addition, when *Escherichia coli* is infected with bacteriophage T7, various host cell proteins, including some ribosomal proteins, are phosphorylated<sup>8</sup>. In our search for possible regulatory changes in the protein synthesising apparatus of virus-infected cells, we have now examined the phosphorylation of ribosomal proteins in HeLa cells infected with vaccinia virus.

Infection of HeLa cells with vaccinia virus caused a fourfold increase in the amount of radioactive phosphate incorporated into the ribosomal proteins of 40S ribosomes (Table 1). There is no difference in the specific radioactivity between 60S ribosomal proteins from uninfected and virus-infected cells. (Radioactivity in the total protein from uninfected 60S subunits cannot be due entirely to contamination of 60S subunits with 40S subunits. We are investigating whether it might be attributed to acidic phosphoproteins.) Subsequent treatment of ribosomal proteins with hot trichloroacetic acid (TCA) and organic solvents (to remove residual contamination due to RNA or phospholipids, respectively) did not substantially affect the difference in specific radioactivity between ribosomal proteins of 40S subunits from infected and uninfected cells.

The proteins of 80S ribosomes were extracted and separated by two-dimensional polyacrylamide gel electrophoresis in conditions which resolve only basic ribosomal proteins (Fig. 1).

**Table 1** Incorporation of radioactivity from <sup>32</sup>P-orthophosphoric acid into the protein of ribosomal subunits from uninfected and vaccinia virus-infected HeLa cells

Experiment	Treatment	<sup>32</sup> P radioactivity in ribosomal protein (c.p.m. µg <sup>-1</sup> )			
		Uninfected		Infected	
		40S	60S	40S	60S
a	Cold TCA	60	72	192	85
b	Hot TCA	42	45	192	69
c	Organic solvents	45	58	179	59

HeLa cells were infected with vaccinia WR virus at 1,000 virions per cell as described<sup>9</sup>. Infected and mock infected cells were suspended after virus adsorption in phosphate-free medium and labelled with <sup>32</sup>P-orthophosphate (50 µCi ml<sup>-1</sup>). After 2.5 h of incubation, cells were washed and homogenised and ribosomal particles were pelleted<sup>10</sup>. The nascent polypeptides were released by treatment with 1 mM puromycin and ribosomes were separated by sucrose gradient centrifugation<sup>10</sup> in 20 mM Tris-HCl, pH 7.5, 500 mM KCl, 3 mM MgCl<sub>2</sub>, 5 mM β-mercaptoethanol. The ribosomal proteins were extracted, dialysed and lyophilised according to the procedure of Sherton and Wool<sup>11</sup>. <sup>32</sup>P-ribosomal proteins were precipitated with (a) 10% TCA at 0 °C, the precipitate was collected after 30 min at 0 °C on glass fibre disks and washed with cold 5% TCA. b, As in a but heated for 20 min at 90 °C in 10% TCA before filtration. c, As in b except that additionally the samples on the disks were extracted with 10 ml of ethanol-ethyl ether (3:1) at 60 °C and as well as with 10 ml of ethyl ether and cold acetone.

Comparison of the stained gels revealed an additional protein located to the left (anodically) of protein S2 in ribosomes from virus-infected cells. Also, the density of staining of protein S2 from infected cells decreased. Autoradiographs of these gels show one phosphoprotein (P1) in uninfected HeLa cells and three phosphoproteins among the ribosomal proteins of virus-infected HeLa cells. Phosphoprotein P1 is common to both types of cells whereas P2 and P3 are only observed with ribosomes of infected cells. These phosphoproteins fulfil satisfactorily the criteria for ribosomal phosphoproteins<sup>13</sup>. (1) Ribosomes have been centrifuged twice through 0.5 M KCl, 0.003 M MgCl<sub>2</sub>. (2) Phosphate is bound covalently to serine and threonine (not shown). (3) The covalently bound phosphate migrates with ribosomal proteins on gels. Further, the treatment with (4) hot TCA and (5) organic solvents showed that the contamination of ribosomal proteins with RNA or phospholipids is negligible.

To estimate quantitatively the amount of phosphate incorporated into individual proteins, proteins were cut out of the gel and <sup>32</sup>P radioactivity was determined. Table 2 shows that spot P1 is labelled in both types of cells whereas regions corresponding to P2 and P3 are not detectably labelled in uninfected cells.

We have undertaken experiments to enumerate the ribosomal proteins of HeLa cells (D. Schiffmann and I. H., unpublished). According to this nomenclature of ribosomal proteins we have identified phosphoprotein P1 as S6 and P2 as S2. We assume that the additional protein spot observed in ribosomes from virus-infected cells (left of the normal position of S2) represents phosphorylated molecules of S2 protein. Its negative phosphate groups cause slower migration to the cathode in the first dimension. The lack of staining of gels in the radioactive

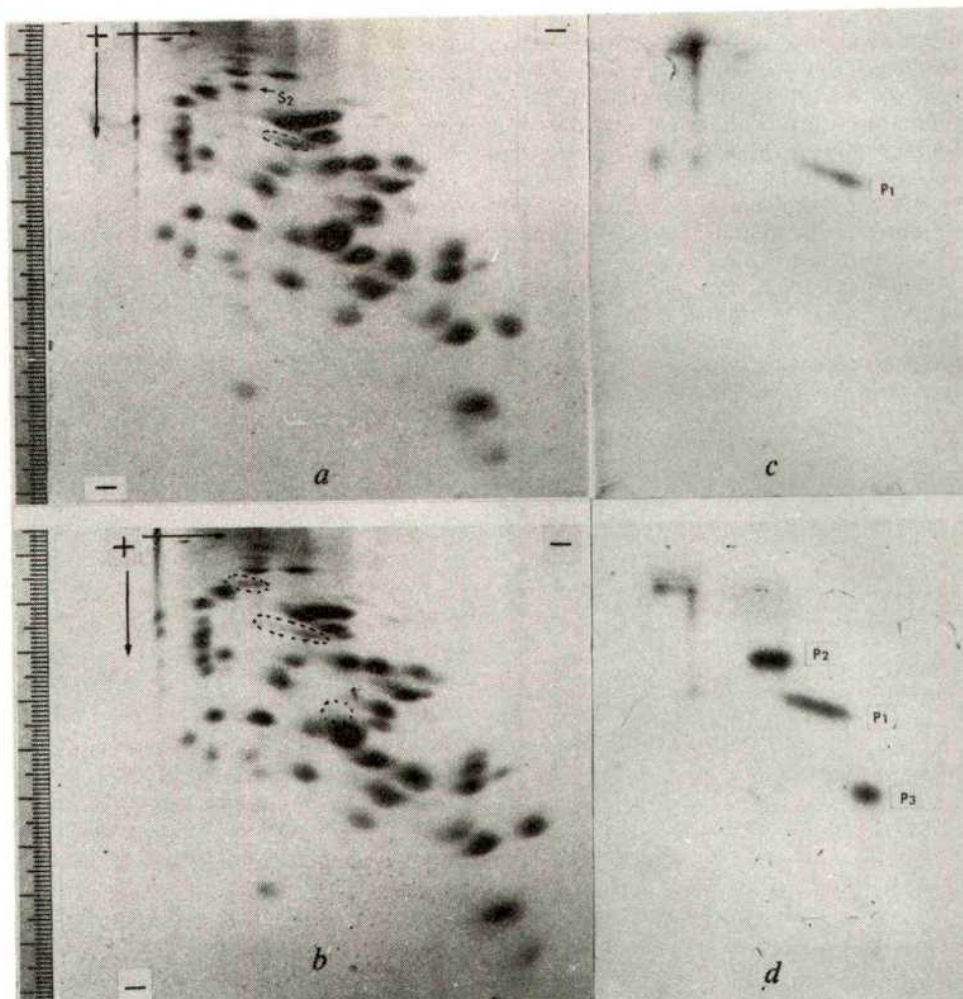
**Table 2** Radioactivity in individual phosphoproteins separated by two-dimensional polyacrylamide gel electrophoresis

Protein	<sup>32</sup> P radioactivity (c.p.m.) in protein from	
	uninfected cells	virus-infected cells
P1 (S6)	871	1,417
P2 (S2)	4	1,809
P3 (?)	0	544

The protein spots were cut out with a cork borer and their radioactivity was determined<sup>13</sup>. The radioactivity in the remaining ribosomal proteins (not shown) did not exceed 25 c.p.m. per spot. This blank value of 25 c.p.m. was subtracted from the values in the table.



**Fig. 1** Two-dimensional electrophoresis of  $^{32}\text{P}$ -ribosomal proteins from 80S ribosomes. Ribosomal proteins were prepared as described in the legend to Table 1. In (a) 820  $\mu\text{g}$  of protein from uninfected cells and in (b) 820  $\mu\text{g}$  of protein from virus-infected cells were separated by two-dimensional polyacrylamide gel electrophoresis<sup>12</sup>, stained with Amido black and photographed. The circles indicate the radioactive areas from the autoradiographs. c, Autoradiograph of the gel a; d, autoradiograph of the gel b. There are no further spots on the autoradiographs beyond the confines of the figure.



area of phosphoprotein P3 suggests a high degree of phosphorylation. Because a high degree of phosphorylation could significantly alter the mobility of a ribosomal protein, it is difficult to ascertain whether or not P3 is a phosphorylated ribosomal, a non-ribosomal cellular or a viral protein.

The mechanism by which ribosomal phosphoproteins arise is not known. Two different reactions, phosphorylation by a kinase and dephosphorylation by a phosphatase, have been considered in the case of protein S6 from rat liver ribosomes<sup>7</sup>. A further possibility might be the phosphorylation of ribosomal proteins by a virion-associated protein kinase, such as that described in vaccinia virions<sup>14</sup> and suggested to be involved in the regulation of host cell functions<sup>15</sup>. Finally, intracellular concentrations of cyclic AMP might be altered in infected cells and consequent effects on the phosphorylation of ribosomal proteins cannot be ruled out.

We cannot say whether the phosphorylation of ribosomal proteins is correlated with functional changes of the translational apparatus caused by the virus. It is tempting to speculate that the phosphorylation of ribosomal proteins is related to the switch off of host cell protein synthesis in virus-infected cells or to the specific selection of viral and cellular mRNAs for translation.

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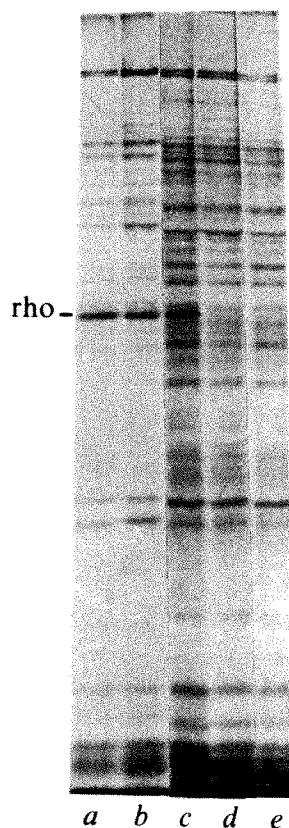
## Evidence that mutations in the *suA* polarity suppressing gene directly affect termination factor rho

THE *suA* mutation in *Escherichia coli* is defined by its suppression of the polar effects of nonsense mutations on distal genes<sup>1</sup>. Richardson *et al.*<sup>2</sup> discovered that two independent mutations in the *suA* gene affect the transcriptional termination factor rho. This suggests<sup>2</sup> a mechanism for polarity: as long as ribosomes progress along the nascent message, transcription proceeds; but when ribosomal progress is blocked, exposure of the distal RNA somehow causes rho to terminate transcription prematurely. As others have noted<sup>3-6</sup>, the involvement of rho in translational polarity could explain several puzzling observations concerning the control of both transcription and translation. Richardson *et al.*<sup>2</sup> proposed that rho may be the product of the *suA* gene. They hesitated to draw that conclusion, however, because they found

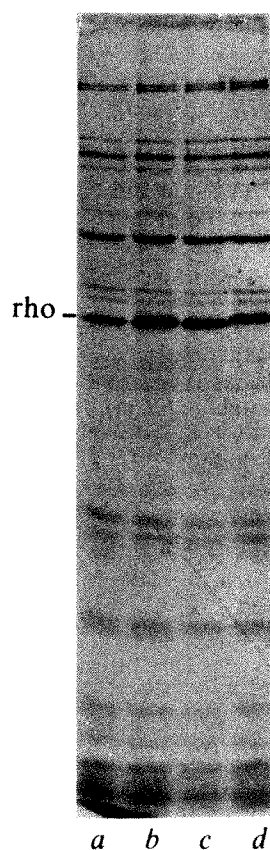


a rather high level of rho activity, 9% of wild type, in a strain believed<sup>7</sup> to contain an amber mutation in the *suA* gene (strain 2055). Either this amber lesion was extraordinarily leaky or, alternatively, the absence of *suA* gene function only partially and indirectly diminished the level of rho activity in the cell. The experiments reported here clarify this problem and argue that the transcriptional termination factor rho is indeed the direct protein product of the *suA* gene.

While examining the binding of bacterial and bacteriophage proteins to affinity resins which contain immobilised *E. coli* RNA polymerase<sup>8</sup>, I noticed that some preparations of immobilised polymerase retained the *E. coli* rho factor.



**Fig. 1** Comparison of the rho polypeptide of *suA* strain 2055 with that of the *su*<sup>-</sup> parent strain 2034. Radioactive cell extracts were prepared by freeze-thaw lysis, digestion of nucleic acids with DNase I and micrococcal nuclease, and brief centrifugation to remove debris (for details, see ref. 8). Extracts (25 µl, corresponding to 1 ml of original cell culture) were applied to affinity columns containing roughly 5 µg of immobilised RNA polymerase. The RNA polymerase holoenzyme used in the affinity resin had been purified through step V of the procedure of ref. 11 and then sedimented down a low salt glycerol gradient; the resulting enzyme is 90–95% pure, as judged by SDS–polyacrylamide gel electrophoresis. The preparation of the affinity resin and the microchromatographic procedures used have been described<sup>8</sup>. The figures show autoradiograms of the 1 M NaCl column elutions analysed on SDS–10% polyacrylamide slab gels<sup>8</sup>. The autoradiograms, using Kodak single-coated blue-sensitive X-ray film, were exposed for 10 d for samples *a* and *b*, and for 7 d for samples *c–e*. *a*, A culture of *su*<sup>-</sup> 2034, grown at 37°C to  $A_{550} = 0.60$  in minimal medium, was pulsed for 7 min with 100 µCi ml<sup>-1</sup> <sup>35</sup>S-methionine, chased briefly (3 min) with excess non-radioactive methionine, and collected on ice<sup>8</sup>. *b*, A parallel culture of *suA* 2055 was treated as above. *c*, A culture of 2034 was grown in minimal medium containing 4 µg ml<sup>-1</sup> <sup>35</sup>SO<sub>4</sub><sup>2-</sup> (New England Nuclear Corp.), 350 µCi ml<sup>-1</sup>. At  $A_{550} = 0.30$ , non-radioactive SO<sub>4</sub><sup>2-</sup>, methionine and cysteine were added in excess to prevent further incorporation of label; the chased cells were then grown for one further generation and collected. *d*, A parallel culture of 2055 was treated as above. *e*, Immediately before loading the affinity resin with a 25 µl sample of the 2034 extract described in *c*, the column was first saturated for rho by the addition of 3 µg of purified rho factor (given by R. Maurer, prepared by the method of Roberts<sup>12</sup>).



**Fig. 2** Analysis of the rho proteins of polarity suppressor strains isolated by M. Malamy. Procedures were as described in Fig. 1. The radioactive pulse was for 7 min, followed by a 3 min chase. Affinity columns contained 15 µg RNA polymerase, and the final autoradiogram was exposed for 2 d. *a*, RV Na1, the *su*<sup>-</sup> parent; *b*, RV Na1 *su*<sup>115</sup>; *c*, RV Na1 *su*<sup>218</sup>; *d*, RV Na1 *su*<sup>221</sup>.

Subsequent experiments have shown that the ability to bind the rho protein is not an intrinsic property of immobilised RNA polymerase, but derives from the presence of a contaminant (possibly rho itself) in some preparations of the polymerase holoenzyme. I have, nevertheless, used these affinity resins to examine the rho protein of several *E. coli* strains. Making use of a slight difference in the molecular weights of the rho polypeptides of strain K12 and B/r, I mapped the rho gene near *ilv* at 75' on the *E. coli* genome<sup>9</sup>. (The observations reported here, in conjunction with the experiments described in ref. 9, make it almost certain that I have identified the structural gene for rho, and not some strain-specific modifier of the rho protein.) Pl co-transduction experiments showed the *rho* gene to be tightly linked to the *suA* locus (see ref. 9); and I was thereby led to examine the rho protein of several polarity suppressor strains.

To observe the rho polypeptide, I prepared crude cell extracts from <sup>35</sup>S-methionine pulsed cultures using freeze-thaw lysis and mild nuclease digestion (see the legend to Fig. 1 for details); the extracts were then applied to affinity columns which were subsequently eluted with a 1 M NaCl buffer. Figure 1 shows the sodium dodecyl sulphate (SDS)–polyacrylamide gel profiles of several of these column elutions. The polarity suppressing mutant 2055, the presumed *suA* amber<sup>2,7</sup>, produces a rho polypeptide of normal molecular weight in amounts comparable with that made by the (*su*<sup>-</sup>) parent strain, 2034, during this pulse (compare columns in Fig. 1*a* and *b*). If the radioactive labelling protocol is changed, however, so that the cells are chased for one generation before collection, then the rho lesion in mutant 2055 becomes apparent: the columns in Fig. 1*c* and

*d* show that the rho protein of the parent strain is relatively stable during the *in vivo* chase, but the rho polypeptide of the polarity suppressed strain can no longer be detected after this treatment. The final column in Fig. 1e demonstrates that the autoradiographic band designated as rho represents this protein: in this experiment, previous addition of excess purified rho to the affinity resin totally and specifically overcame the binding of the radioactive rho protein in the 2034 extract.

The observation of wild-type levels of the full-sized rho polypeptide in pulsed extracts of *suA* mutant 2055 is not compatible with the assumption that 2055 harbours an amber mutation in the *rho* gene. On the contrary, the data of Fig. 1 suggest that 2055 may be a missense mutation resulting in an unstable rho protein. Genetic experiments confirmed that the *suA* phenotype of 2055 is not (currently) due to an amber mutation. Following the method which Morse and Guertin<sup>7</sup> used to detect the original amber mutation, I introduced the *su3* amber suppressor into strain 2055 (of genotype<sup>7</sup> *W3110 tryp E' ochre 9851, leu<sup>-</sup>amber 277, suA 120*) with  $\phi 80\text{psu3}$ , selecting *leu<sup>+</sup>* lysogens and then scoring for the continued expression of the *suA* allele. Each colony tested (22 independent isolates from the 2055 stab) continued to express its *suA* phenotype even in the presence of the *su3* amber suppressor. Examination of other strains presumed to carry the *suA* amber allele (2054 from D. Morse, and CU111 (a derivative of 2054) from H. Umbarger<sup>10</sup>) revealed no cells with true *suA* amber mutations. D. Morse and J. Richardson (personal communications) confirm that now the *suA* phenotype of these strains is not the result of an amber mutation in that locus. Since the *suA* lesion in strain 2055 is a missense mutation producing an unstable rho protein, the 9% level of activity detected by Richardson *et al.*<sup>2</sup> is in complete agreement with the contention that rho is the primary gene product of the *suA* locus.

I have screened the rho proteins of another set of polarity suppressed mutants, those isolated by M. Malamy (in preparation) for their effect on insertion mutations in the lactose operon<sup>13</sup>. The polarity suppressing mutations map at 75' and are presumed to lie in the locus originally designated *suA*. Figure 2 shows that three of these mutations do affect rho. In mutant 221, the rho polypeptide is of slower gel mobility (presumably greater molecular weight) than normal. The simplest interpretation of the 221 effect is that the *suA* mutation lies in the structural gene for rho. Each of the *suA* strains seems, by this method of detection, to overproduce the rho protein. Mutant 115, the strongest polarity suppressor in that collection (M. Malamy, personal communication), is the greatest overproducer. Densitometry of the autoradiograms indicates that pulsed cultures of strain 115 contain at least three times as much radioactivity in the rho polypeptide as the RV Nal parent strain. (Even after a one generation chase, strains 221 and 115 display at least as much radioactive rho as the parent strain.) It is surprising that these polarity-suppressing strains, presumed to contain diminished rho activity, seem to overproduce the rho polypeptide. This may be an indication of autoregulation of rho synthesis, conceivably the result of a rho-dependent attenuation of transcription<sup>14</sup> of the *suA* gene.

The *suA* mutations described here affect rho in disparate ways: strain 2055 makes an unstable rho; other strains, especially *su<sup>115</sup>*, overproduce rho; and *su<sup>221</sup>* apparently increases the molecular weight of the polypeptide. There are other mutations, linked to *ilv*, that affect specific termination processes. The mutants of Bertrand *et al.*<sup>14</sup>, which suppress attenuation of the tryptophan operon, also suppress polarity, but the *sun* mutants described by Brunel and Davison<sup>15</sup>, relieving the N requirement of phage  $\lambda$ , do not. The widely differing phenotypes of all these mutants are probably a consequence of the necessarily subtle nature of

mutations allowed in rho, reflecting the essential role that rho must have in bacterial transcription.

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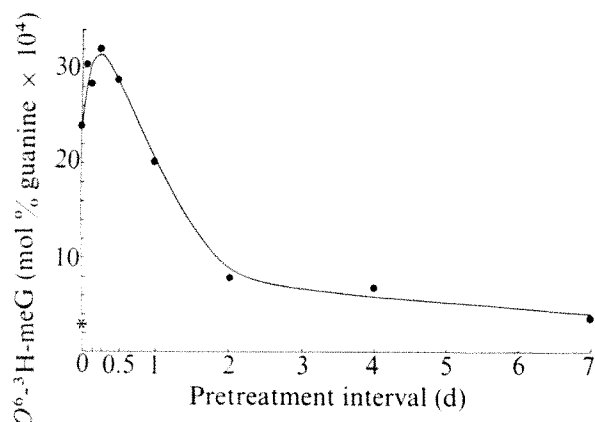
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## Exhaustion and recovery of repair excision of *O*<sup>6</sup>-methylguanine from rat liver DNA

EVIDENCE continues to accumulate that the *O*<sup>6</sup> position of guanine in DNA is a critical reaction site for the initiation of malignant transformation by monofunctional alkylating agents. *O*<sup>6</sup>-alkylguanine has been shown to be promutagenic and the relative extent of its formation by different compounds approximately corresponds to their carcinogenic potency<sup>1-3</sup>. In contrast to 7-alkylguanine, which is the major reaction product in nucleic acids, *O*<sup>6</sup>-alkylguanine can be enzymically excised from DNA<sup>4-7</sup> and the tissue-specific carcinogenic action of alkylating carcinogens seems to depend on the differential capacity of the various organs to actively remove this base from their DNA<sup>8-11</sup>. The principal target organ in the carcinogenicity of *N*-methyl-*N*-nitrosourea (MNU) is the nervous system and it has been shown that *O*<sup>6</sup>-methylguanine (*O*<sup>6</sup>-meG) is removed much less rapidly from brain DNA than from that of any other rat tissue<sup>6,11</sup>. The liver is not susceptible to the carcinogenicity of MNU (except after partial hepatectomy<sup>12</sup>) and this organ has been found to be most efficient in the repair excision of *O*<sup>6</sup>-meG (ref. 11). We have noted<sup>9</sup>, however, that the loss of *O*<sup>6</sup>-meG from liver DNA is influenced markedly by the dose of MNU administered; higher levels of *O*<sup>6</sup>-meG are less rapidly removed. We now present evidence that the excision repair system for *O*<sup>6</sup>-meG in rat liver can be overloaded with sublethal doses of MNU and related carcinogens, and requires several days to recover and restore its initial capacity.

In all experiments, female BD-IX rats (110-140 g) received an intravenous injection of 10 mg kg<sup>-1</sup> <sup>3</sup>H-MNU and were killed 6 h later. Liver DNA was isolated and purine bases were separated by Sephadex G-10 chromatography of the acid hydrolysate<sup>11</sup>. After injection of <sup>3</sup>H-MNU alone *O*<sup>6</sup>-<sup>3</sup>H-meG present in liver DNA 6 h later amounted to 3.3 × 10<sup>-4</sup> mol % of guanine (Fig. 1). When unlabelled MNU (70 mg kg<sup>-1</sup>) was administered simultaneously with, or up to 12 h before, the injection of <sup>3</sup>H-meG the amount of *O*<sup>6</sup>-<sup>3</sup>H-meG present 6 h later was 7-10 times higher, indicating that it was excised from DNA at a considerably lower rate. The excision capacity of the liver gradually recovered during longer pretreatment intervals but even 7 d after the injection of unlabelled MNU the initial rate

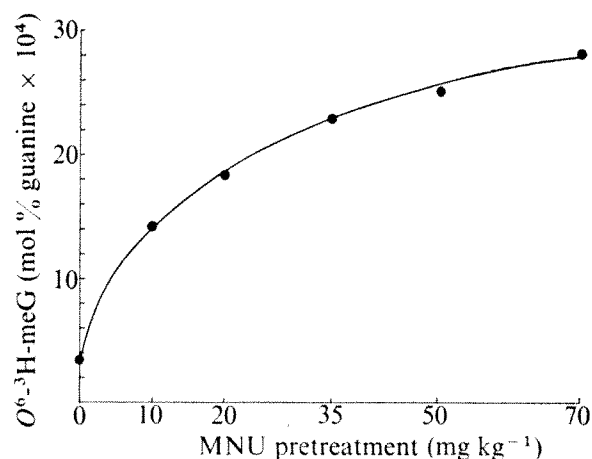


**Fig. 1** Amounts of  $O^6$ - $^3H$ -meG present in liver DNA 6 h after an intravenous injection of  $10 \text{ mg kg}^{-1}$   $^3H$ -MNU. Unlabelled MNU ( $70 \text{ mg kg}^{-1}$ ) was administered either simultaneously or at various times before injection of  $^3H$ -MNU (pretreatment interval). Asterisk on ordinate indicates amount of  $O^6$ - $^3H$ -meG present when no additional unlabelled MNU was administered. DNA was isolated by phenol extraction from the pooled livers of two rats and hydrolysed in  $0.1 \text{ M HCl}$  ( $37^\circ\text{C}$ , 20 h). Purine bases were determined after chromatography on Sephadex G-10 columns ( $1 \times 90 \text{ cm}$ ) eluted with  $0.05 \text{ M}$  ammonium formate ( $\text{pH } 6.8$ ). Extent of methylation was calculated from specific radioactivity of injected  $^3H$ -MNU ( $12.5 \text{ mCi mmol}^{-1}$ ). For details see ref. 11.

of  $O^6$ - $^3H$ -meG excision was not yet fully re-established. The effect of pretreatment with various doses of unlabelled MNU on the amount of  $O^6$ - $^3H$ -meG persisting in liver DNA is shown in Fig. 2. In this experiment, a constant pretreatment interval of 3 h was chosen. The results provide no evidence for a threshold effect: even pretreatment with a dose of only  $10 \text{ mg kg}^{-1}$  MNU significantly increased the amount of  $O^6$ - $^3H$ -meG present 6 h after the administration of  $^3H$ -MNU.

To test the specificity of the pretreatment effect, sublethal doses of X rays and agents of various carcinogenic potency were applied (Table 1). Compounds known to produce a high extent of alkylation in the  $O^6$  position of guanine (ethylnitrosourea, dimethylnitrosamine<sup>3</sup>) markedly reduced the excision of  $O^6$ - $^3H$ -meG produced by the subsequent injection of  $^3H$ -MNU. Methyl methanesulphonate was given at a dose ( $85 \text{ mg kg}^{-1}$ ) known to produce approximately the same total extent of alkylation of DNA as  $70 \text{ mg kg}^{-1}$  MNU<sup>13</sup>. The effect of methyl methanesulphonate pretreatment on the excision of  $O^6$ - $^3H$ -meG, however, was equivalent to a dose of only about  $4 \text{ mg kg}^{-1}$  MNU (Table 1, Fig. 2). This could be explained by the fact that  $4 \text{ mg kg}^{-1}$  MNU would be expected to produce approximately the same amount of  $O^6$ -meG as  $85 \text{ mg kg}^{-1}$  methyl methanesulphonate<sup>3,14</sup>. At the same time, the results of methyl methanesulphonate

administration indicate that the pretreatment effect on  $O^6$ -meG excision is indeed mediated by the production of  $O^6$ -alkylguanine itself rather than alkylation products such as 7-alkylguanine. The other agents used for pretreatment (Table 1) are not known to produce  $O^6$ -alkylation of guanine. The small effects observed after pretreatment with *N*-nitrosomorpholine, 7,12-dimethylbenz [*a*] anthracene and cyclophosphamide suggest that these agents may only to a limited extent produce DNA lesions which are recognised and repaired by the  $O^6$ -meG excision system. Ethionine, like nitrosomorpholine a potent liver carcinogen, reacts with DNA to only a very small extent<sup>15</sup> and was found to have no effect on the excision of  $O^6$ - $^3H$ -meG from liver DNA. X irradiation was the only pretreatment which caused a more rapid excision, although to only a small extent.



**Fig. 2** Effect of various pretreatment doses of MNU on excision of  $O^6$ - $^3H$ -meG from rat liver DNA *in vivo*. Animals received  $10$ – $70 \text{ mg kg}^{-1}$  unlabelled MNU. After an interval of 3 h, they were given  $^3H$ -MNU ( $10 \text{ mg kg}^{-1}$ ) and killed 6 h later. For experimental details, see Fig. 1.

The results obtained cannot be explained by an increase in the overall rate of alkylation attributable to the pretreatment since this would proportionally lead to an increase in alkylation at the 7 position of guanine. Only minor changes in the  $7$ - $^3H$ -meG levels, however, were found: the decrease in the  $O^6$ -meG excision was evident from an increase in both the absolute amounts of  $O^6$ - $^3H$ -meG and the  $O^6$ -/ $7$ - $^3H$ -meG ratio (Table 1).

The present study shows that rat liver may efficiently excise  $O^6$ -alkylguanine produced by a single dose of an alkylating carcinogen but that a second dose, given after an interval of less than about 12 h, produces additional DNA damage which is much less efficiently repaired. This may correspond to the observation that liver carcinogens in general do not produce tumours when given as a single

**Table 1** Effect of pretreatment with various agents on excision of MNU-induced  $O^6$ -meG from rat liver DNA

Pretreatment	Dose	Route	$O^6$ - $^3H$ -meG*	Ratio $O^6$ -/ $7$ - $^3H$ -meG
Control	—	—	3.3	0.018
X irradiation†	400 R	—	1.8	0.011
DL-Ethionine	$750 \text{ mg kg}^{-1}$	i.p.	3.2	0.023
Cyclophosphamide	$90 \text{ mg kg}^{-1}$	i.v.	4.3	0.031
7,12-Dimethylbenz [ <i>a</i> ] anthracene	$100 \text{ mg kg}^{-1}$	i.p.	6.4	0.029
<i>N</i> -nitrosomorpholine	$50 \text{ mg kg}^{-1}$	i.v.	7.5	0.039
Methyl methanesulphonate	$85 \text{ mg kg}^{-1}$	i.v.	10.4	0.054
<i>N</i> -ethyl- <i>N</i> -nitrosourea	$120 \text{ mg kg}^{-1}$	i.v.	18.9	0.086
MNU (cold)	$70 \text{ mg kg}^{-1}$	i.v.	28.3	0.120
<i>NN</i> -dimethylnitrosamine	$20 \text{ mg kg}^{-1}$	i.p.	32.3	0.125

\* $O^6$ - $^3H$ -meG in liver DNA, expressed as  $\text{mol \%} \times 10^4$  of guanine.

†Whole body irradiation using a  $^{60}\text{Co}$  source.

Animals received an intravenous injection of  $10 \text{ mg kg}^{-1}$   $^3H$ -MNU and were killed 6 h later. Pretreatment with equitoxic doses (approximately 50% of the acute  $\text{LD}_{50}$ ) of various agents was carried out 3 h before injection of  $^3H$ -MNU. i.p., intraperitoneal; i.v., intravenous.



dose but are most effective after daily administration in the diet or drinking water<sup>16</sup>. The pretreatment scheme used in this study may be useful to test whether a carcinogen produces DNA lesions which saturate the *O*<sup>6</sup>-alkylguanine excision system by competing for the same repair enzymes.

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## Altered C-terminal salt bridges in haemoglobin York cause high oxygen affinity

STUDIES of abnormal human haemoglobins (Hbs) with increased  $O_2$  affinity have been particularly useful in developing detailed molecular interpretations of normal Hb function. A number of functionally interesting mutants are altered in the  $\beta$ HC region but only one variant, Hb Hiroshima ( $\beta$ 146 His $\rightarrow$ Asp)<sup>1,2</sup>, has been reported as having a substitution at the C terminus itself. We report here the structure and  $O_2$  equilibria of Hb York, a new mutation at the  $\beta$ 146 position, His $\rightarrow$ Pro, associated with increased  $O_2$  affinity, decreased cooperativity and diminished Bohr effect.

Hb York was discovered during investigation of mild asymptomatic erythrocytosis in a 22-yr-old Caucasian woman. Preliminary measurements showed markedly increased whole blood  $O_2$  affinity, suggesting the presence of an abnormal Hb. Although Hb electrophoresis on starch gel or on cellulose acetate at alkaline pH failed to reveal an abnormal band, two major components were separated on agar gel electrophoresis at pH 6.0 in 0.05 M citrate buffer<sup>3</sup>. Hb York migrated more rapidly towards the cathode than Hb A and constituted approximately 50% of total Hb.

Hb York was separated from Hb A on carboxymethyl-cellulose<sup>3</sup>, and was shown to be homogeneous on agar gel electrophoresis. The presence of approximately equal amounts of Hb A and York suggested that Hb York was a  $\beta$  mutant. The fractions containing Hb York in the CO form were concentrated by pressure ultrafiltration and dialysed exhaustively against 0.1 M NaCl.

Purified Hb York was converted to globin with cold acid acetone<sup>4</sup>. The abnormal  $\beta$  chain was isolated by the method of Clegg *et al.*<sup>5</sup>. Amino acid analysis of the HCl-hydrolysed  $\beta$  chain showed a low value for histidine and a high value for proline compared with  $\beta^A$  chains. The  $\beta^{\text{York}}$  chains were aminoethylated as described previously<sup>6</sup> and digested with trypsin<sup>7</sup>. The tryptic peptides were chromatographed on Aminex A-5 (ref. 8), and the chromatogram appeared similar to that resulting from a tryptic digest of  $\beta^A$  chains except that the  $\beta$ T-15 (Tyr–His) was absent. Rechromatography on Aminex AG 50W-X4 of a fraction containing the  $\beta$ T-4 and an abnormal peptide gave a good yield of a peptide having the amino acid

composition shown in Table 1. This corresponds to the normal  $\beta$ T-14,15 peptide except that a histidine has been replaced by proline. This substitution was confirmed by analysis of the peptides resulting from partial hydrolysis of the  $\beta$ T-14, 15 peptide by thermolysin (Calbiochem, Crystalline B Grade)<sup>9</sup>. Analysis of the other tryptic peptides following HCl hydrolysis revealed them to have normal composition.

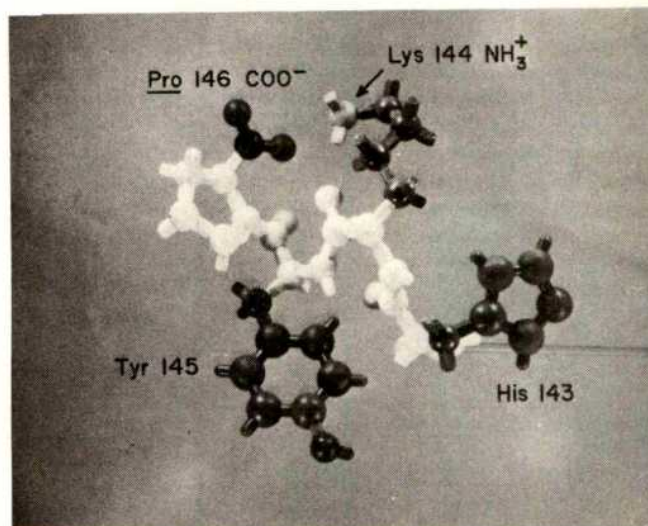
To distinguish between mutation of the  $\beta$ 146 and  $\beta$ 143 histidine residues, tryptic hydrolysis of the  $\beta$  chain after glycinamidation by the method of Carraway and Koshland<sup>10</sup> was carried out. A normal  $\beta$ T-14 peptide was obtained in good yield, indicating that the mutation was at the  $\beta$ 146 rather than the  $\beta$ 143 histidine. The glycinamidated  $\beta$ T-15 (Tyr–Pro) peptide was not observed, presumably owing to the degrading effect of glycinamidation on tyrosine residues.

Carboxypeptidase A digestion of  $\beta^A$  and of  $\beta^{\text{York}}$  chains was carried out according to Ambler<sup>11</sup> in 2 M urea at pH 8.5 for 2 h at 37 °C. Although both Tyr and His were removed from the  $\beta^A$  chain, no amino acid residues were removed from  $\beta^{\text{York}}$ . Proline at the C terminus would be expected to resist the enzyme. Hb York is therefore  $\beta$ 146 (His $\rightarrow$ Pro).

Functional studies of Hb York revealed a very high  $O_2$  affinity and diminished Bohr effect and cooperativity. The  $\log P_{50O_2}$  of Hb York was 0.04 in contrast to 0.71 for similarly prepared Hb A, measured at 20 °C in 0.1M phosphate, pH 7.0. The alkaline Bohr effect of Hb York was approximately 50% normal ( $\Delta \log P_{50}/\Delta pH = -0.36$  compared with  $-0.65$  for Hb A). Hill's '*n*' for Hb York is 1.8 ( $\pm 0.2$ ) compared with 2.5 for Hb A in these conditions, and did not change with pH. The effect of the organic phosphates, 2,3 diphosphoglycerate (DPG) and inositol hexaphosphate (IHP), on the  $O_2$  equilibria of Hb York and Hb A is shown in Table 2. DPG and IHP are known to bind to the  $\beta$  chains and stabilise the quaternary conformation of deoxy Hb, thus lowering  $O_2$  affinity. The changes of  $\log P_{50O_2}$  of Hb York produced by DPG and by IHP are similar to those observed with Hb A. In contrast to the diminution of Hill's '*n*' to 1.6 observed with Hb A in the presence of IHP, the value for Hb York was increased from 1.8 to 2.2. The visible and ultraviolet spectra of Hb York were similar to those of Hb A.

Hb York may be contrasted with Hb Hiroshima ( $\beta$ 146, His $\rightarrow$ Asp)<sup>2</sup>. Both mutants have a similarly reduced Bohr effect owing to loss of the intrachain salt bridge between the  $\beta$ 146 histidyl imino  $NH^+$  and the  $\beta$ 94 aspartyl carboxylate group. The Bohr effect of des-(His- $\beta$ 146) Hb A has also been shown to be diminished<sup>13</sup>. The  $O_2$  affinity of Hb York is,

**Fig. 1** Model of C-terminal region of  $\beta$  chain of deoxy Hb York showing proposed salt bridge between  $\beta$ 146 Pro carboxylate and  $\epsilon$ -amino group of  $\beta$ 144 lysine. Constructed from coordinates of horse deoxy Hb except for the inclusion of prolyl residue and rotation of lysine side chain.





**Table 1** Composition of C-terminal peptides of  $\beta$  chain of Hb York (amino acid residues per haem)

Amino acid	Tryptic $\beta$ T-14,15	Thermolysin peptide from $\beta$ T-14,15	Glycinamidated tryptic $\beta$ T-14
Proline	1.0(0)*	1.0(0)*	—(0)*
Tyrosine	0.8(1)	1.0(1)	—(0)
Histidine	1.0(2)	0.9(2)	1.0(1)
Lysine	1.1(1)	1.1(1)	1.1(1)
Alanine	4.0(4)	1.0(1)	3.7(4)
Leucine	1.1(1)	1.0(1)	1.0(1)
Aspartic acid	1.1(1)	—	1.1(1)
Valine	3.0(3)	—	2.9(3)
Glycine	1.0(1)	—	1.0(1)

\* Values in parentheses are those expected for corresponding peptides in normal Hb.

however, significantly higher than that of Hb Hiroshima or of des-(His- $\beta$ 146) Hb A (Table 3). Hill's ' $n$ ' is lower than that observed for either of these Hbs.

The mechanism for the abnormal  $O_2$  affinity of Hb York may involve destabilisation of its 'T' conformation. This destabilisation might be due to loss not only of the intrachain salt bridge between the  $\beta$ 146 His  $NH^+$  and the  $\beta$ 94 Asp carboxylate but also to impaired formation of the interchain salt bridges between the  $\beta$ 146 carboxylate and  $\alpha$ 40 Lys  $\epsilon$ -amino group which is characteristic of the normal T conformation of deoxy Hb<sup>14</sup>. Hb Hiroshima does not lose the latter salt bridge<sup>2</sup>. We constructed a model of the C-terminal region of the  $\beta$  chain of horse deoxy Hb, using energy refined coordinates supplied by M. F. Perutz. This model indicated that formation of the

salt bridges which contribute to the stabilisation of the deoxy conformation of Hb A.

Crystals of deoxy des-(His- $\beta$ 146) Hb A have also been described<sup>15</sup> as lacking the four  $\beta$ -chain salt bridges, yet the functional properties of this haemoglobin are more similar to those of Hb Hiroshima than Hb York. The modifications to the T  $\rightleftharpoons$  R equilibrium of the des-(His- $\beta$ 146) derivative seem to be more complex<sup>16</sup> than in Hb York.

The loss of the four  $\beta$ -chain C-terminal salt bridges is not sufficient to prevent deoxy Hb York from entering the T conformation because it possesses a significant degree of cooperativity, because the effects of DPG and IHP on its  $O_2$  affinity are similar to those for Hb A, and because the visible and ultraviolet spectra of deoxy Hb York are very similar to those of deoxy Hb A. The degree of destabilisation of the T conformation proposed for Hb York, however, would be greater than that occurring in Hb Hiroshima due to the loss of the interchain salt bridges. This additional destabilisation would account for the more pronounced abnormalities of  $O_2$  binding of Hb York.

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**Table 2** Effects of organic phosphates on  $O_2$  equilibria\* of Hb York and Hb A

	$\log P_{50O_2}$	Hill's $n$	$\Delta \log P_{50O_2}^\dagger$
Hb York			
'Stripped'	-0.21	1.8	—
0.2 mM DPG	-0.02	2.0	0.19
1.0 mM IHP	0.70	2.2	0.91
Hb A $^\ddagger$			
'Stripped'	0.67	2.6	—
0.2 mM DPG	0.90	2.5	0.23
1.0 mM IHP	1.63	1.6	0.96

\*  $O_2$  equilibria determined at pH 7.0 and 20 °C using a modified<sup>3</sup> technique of Benesch *et al.*<sup>12</sup>. Samples were buffered with 0.05 M bis-Tris and contained 0.1 M chloride. Formation of methaemoglobin during these experiments did not exceed 5% of the total.

$^\dagger \Delta \log P_{50O_2} = \log P_{50O_2} - \log P_{50O_2}$  (Stripped Hb).

$^\ddagger$  Hb A was prepared by passing a haemolysate from normal human adult red cells through Sephadex G-25 equilibrated with 0.1 M NaCl.

$\beta$ 146- $\alpha$ 40 salt bridge is seriously hindered in Hb York. The referee has kindly stated that "The reason why the salt bridge between the  $\alpha$ -carboxyl of Pro- $\beta$ 146 and Lys- $\alpha$ 40 is not formed is that the Pro is too close to the Cys- $\beta$ 93." The  $\beta$ 146 proline residue and the side chain of the  $\beta$ 144 lysine can, however, be readily oriented (Fig. 1) so that an intrachain salt bridge is formed between the  $\beta$ 146 carboxylate and the  $\epsilon$ -amino group of  $\beta$ 144 lysine. Formation of this salt bridge does not alter the position of the critical  $\beta$ 145 tyrosine. We suggest that this salt bridge would not be broken by transition to the 'R' conformation on oxygenation and, therefore, would not contribute to the free energy of interaction. We thus propose that the deoxy conformation of Hb York lacks the four  $\beta$ -chain C-terminal

**Table 3** Comparison of  $O_2$  equilibria\* of Hb York with Hb Hiroshima and des-(His- $\beta$ 146) Hb A

	Hb York	Hb Hiroshima	des-(His- $\beta$ 146) Hb A
$\log P_{50O_2}$	0.04	0.3	0.28
Hill's $n$	1.8	2.0	2.5
Bohr effect	Decreased	Decreased	Decreased
Reference	This paper	1	13

\* Properties determined at or corrected to pH 7.0, 20 °C in 0.1 M phosphate buffer.

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# matters arising

## Weak interactions in the big bang

THE apparent arbitrariness of the numerical values assumed by the various constants of physical theory has from time to time led to the conjecture that these quantities may not be fixed numbers at all, but vary from epoch to epoch as the Universe evolves. Such a suggestion was advanced, on the basis of some discussion about gauge theories and spontaneous symmetry breaking, by Domokos, Janson and Kövesi-Domokos<sup>1</sup> in connection with the weak interaction coupling strength. They propose that this coupling strength was comparable with electromagnetism during the primordial fireball phase of the big bang (specifically, before  $T \approx 10^5$  K). This is some  $10^{10}$  times greater than at present, and its consequences are being investigated by the authors.

One consequence which they will discover is that, on general grounds, the lifetime of the neutron against  $\beta$  decay,  $n \rightarrow p + e^- + \bar{\nu}$ , would presumably be reduced by the same factor of  $\sim 10^{10}$ , making the half life of neutrons in the fireball phase a mere  $10^{-7}$  s or so. The implications of this are profound. According to current models of the hot big bang, protons and neutrons are maintained in thermodynamic equilibrium by electron-positron processes until the end of the lepton era at  $\sim 1$  s ( $10^{10}$  K). After this, the electron-positron pairs annihilate, and the neutron/proton abundance ratio is thereafter 'frozen in' until the temperature drops sufficiently low (about  $10^9$  K) for helium synthesis to occur. Essentially all the remaining neutrons are incorporated into helium (where they remain stable) after a few hundred seconds, which is well within the currently measured lifetime of the neutron.

Accepting the model of Domokos *et al.* would lead to the conclusion that the neutron abundance would not remain frozen at all during the intermediate cooling phase, but would decay to virtually zero long before nucleosynthesis could begin. The consequences of that would be an absence of neutrons in the universe—indeed, a universe composed entirely of hydrogen!

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DOMOKOS ET AL. REPLY—DAVIES' remark<sup>1</sup> concerning our recent letter<sup>2</sup> raises a relevant issue; in fact, we have been studying this problem for some time. If interpreted literally, our result may lead to the paradox pointed out by Davies. The careful reader will, however, have noticed that in order to illustrate the basic idea expressed in our letter (generation of a distinction between weak and e.m. interactions through a cosmological background) we took an extremely crude idealisation of an expanding universe. In fact, our input scalar curvature is:  $R/(3p - \rho) = 0$  ( $x < x_0$ ) and  $R \sim x^{-3}$  ( $x > x_0$ ). In such a universe the temperature drops abruptly from infinity to essentially 'zero' at  $x_0$ . Hence, for  $x < x_0$  there is no nucleus formation and no neutron decay either (due to an infinite time dilatation factor). All this can take place only for  $x > x_0$ . Then  $\phi(x)$  rises very rapidly and gets close to its present value quite soon. Thus, even in this highly idealised model, there seems to be no qualitative contradiction with usual ideas of He formation. On the basis of computer experiments performed with more realistic models, we are now under the impression that the general shape of the curve  $\phi(x)$  is correctly given by our model<sup>2</sup>. Davies' observation imposes a consistency condition on more realistic model calculations:  $\phi$  must come out to be substantially different from zero during the epoch of primordial He formation.

We thank P. C. W. Davies for informing us about his observations before publication. This research is being supported by the US Energy Research and Development Administration.

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<sup>2</sup> Domokos, G., Janson, M. M., and Kövesi-Domokos, S., *Nature*, **257**, 203–204 (1975).

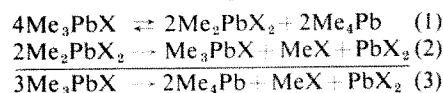
## Methylation of organolead and lead(II) compounds to $(CH_3)_4Pb$ by microorganisms

RECENTLY evidence<sup>1</sup> showed that microorganisms can transform certain lead compounds into  $Me_4Pb$ ; addition of  $Me_3PbOAc$  to sediment samples apparently always increased the production of  $Me_4Pb$ . The addition, of lead(II) compounds did not, however, lead to definite effects, and with pure bacterial isolates no transformation of  $Pb^{2+}$  to  $Me_4Pb$  could be observed<sup>1</sup>. Doubt has since been cast<sup>2</sup> on the significance of a microbiological conversion of  $Me_3PbOAc$  to  $Me_4Pb$ , preferring a chemical mechanism;

also alkylation of  $Pb(NO_3)_2$  could not be detected (ref. 2).

Our studies of the behaviour of lead compounds in the presence of microorganisms show that  $Pb^{2+}$  can be biologically alkylated to  $Me_4Pb$ , and this helps to explain the formation of  $Me_4Pb$  from  $Me_3PbOAc$  in anaerobic systems.

In aqueous solution, alkyl lead compounds  $R_2PbX_2$  and  $R_3PbX$  ( $X = \text{anion}$ ) redistribute according to



reaction (2) is much faster than (1)<sup>3,4</sup> and therefore  $Me_3PbX$  seems to be relatively stable at room temperature. The stoichiometry of its decomposition can be described practically by reaction (3).

In water samples (dilution water<sup>5,6</sup>) seeded with microorganisms from an aerated aquarium, we observed, apart from the inhibition of biodegradation, that di-alkyl and tri-alkyl lead concentrations decreased considerably faster in both aerobic and anaerobic conditions than in sterile samples, and that the proportions of products no longer agrees with predictions from the above system of equations. After addition of  $Me_3PbCl$  to an anaerobic sample, less  $Pb^{2+}$ , but more  $Me_4Pb$  was formed than is required by reaction (3). Furthermore, the amount of  $Me_4Pb$  produced in a given time was considerably higher than from a sterile sample (by a factor of 5–10 in a week). Therefore the possibility that  $Me_4Pb$  was produced only by redistribution (reaction (1)), could be excluded, and it was an obvious assumption that  $Me_3PbCl$  and/or  $Pb^{2+}$ , formed according to reaction (3), had been alkylated by microorganisms to  $Me_4Pb$ .

We then investigated whether  $Pb^{2+}$  could be alkylated by microorganisms in the same anaerobic conditions. We inoculated water samples containing  $Pb(OAc)_2$  in different low concentrations (Table 1) and sealed the flasks after flushing with  $N_2$ . The analysis for  $Me_4Pb$  (the only volatile lead compound in the system) was carried out by slowly sweeping the gas phase above the solution with  $N_2$  through an 0.2 N methanolic iodine scrubber solution<sup>7</sup>; the lead content was determined colorimetrically with 4-(2-pyridylazo)-resorcinol<sup>8</sup>. The experiments were reproducible, provided that the seed was not more than 6–7 weeks old.

We therefore conclude, that besides the formation of  $Me_4Pb$  by redistribution according to reaction (1), biological alkylation of  $Pb^{2+}$  must be considered as

<sup>1</sup> Domokos, G., Janson, M. M., and Kövesi-Domokos, S., *Nature*, **257**, 203–204 (1975).

Table 1 Methylation of Pb<sup>2+</sup> by microorganisms

Pb <sup>2+</sup> Concentration* ( $\mu\text{g ml}^{-1}$ )	Seed type†	Incubation time (d)	Pb( $\mu\text{g}$ )‡ analysed	MePb( $\mu\text{g}$ )§ produced
10	I	7	6	8
1	I	7	12	15
0.1	I	7	~10	~13
1	II	14	82	106
1	II	7	80	103
		(+7)	(+10)	(+13)
1	II	7	56	72
		(+7)	(+20)	(+26)

\*Starting concentration of Pb(OAc)<sub>2</sub> in a seeded 100-ml water sample (nutrient: glucose; seed type I or II) in a 250-ml gas wash bottle.

†Seeds were prepared from 10 ml A-water (= water from an aerated aquarium), 200 mg glucose and 10 mg urea, made up with water to 100 ml, and then incubated under N<sub>2</sub>. Seed type I: 10 ml seed (incubated for 2 weeks) + 10 ml seed (incubated for 6 weeks) + 10 ml A-water. II: 10 ml seed (incubated for 5 d) + 10 ml A-water.

‡Volatile Pb found in scrubber solution (see text).

§Amount of Me<sub>4</sub>Pb equivalent to analysed amount of Pb.

|| Same solution, additional incubation time in N<sub>2</sub>, after exchange of gas atmosphere, giving additional quantities of reaction product.

another source for Me<sub>4</sub>Pb in the experiments with Me<sub>3</sub>PbOAc (refs 1, 2). (The possibility of direct methylation of Me<sub>3</sub>PbOAc still has to be investigated.) Furthermore one can expect that the portion of Me<sub>4</sub>Pb chemically formed by redistribution is higher in sulphide systems<sup>2</sup>, as the redistribution rate greatly increases with increasing concentration of added salt MX and with increasing polarisability of X<sup>3,4</sup>. Also, since Pb<sup>2+</sup> formed according to reactions (2) or (3) is precipitated as PbS, not enough Pb<sup>2+</sup> is in solution to allow appreciable microbial alkylation to Me<sub>4</sub>Pb.

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## Muscle regeneration in dystrophic mice

In their letter<sup>1</sup>, Hamburgh *et al.* set out to show that other workers in their field<sup>2</sup> had drawn incorrect conclusions from scanty experimental evidence. There are, however, certain inaccuracies in their text which do not do anything towards their claim of clarification.

In one paragraph we read that, “foetal cord from 15-d-old mouse embryos . . . were explanted”. A little later we read, “spinal cords were dis-

sected from foetal mice aged 13–14 d *in utero*”. This may seem a trivial point, but the foetal age is critical in these experiments, and such factual mistakes could lead other workers seriously astray.

Their most serious error is in the confusion of the two allelic mutant genes *dy*<sup>2</sup>*J* and *dy*. Their experiments were carried out with the *dy*<sup>2</sup>*J* mutant exclusively. They state, however, that, “normal muscle coupled with either normal or *dy* foetal spinal cord regenerated in culture”. One can only assume that these authors were using ‘*dy*’ as an abbreviation for dystrophic, or else they are guilty of negligence. Whichever applies, it seriously detracts from the value of the work.

They have also misinterpreted my letter<sup>3</sup> in which I did not confirm their work because my experimental system was entirely different. I did not say that “dystrophic” muscle would regenerate normally, since of the two mutants I described, only one (*dy*<sup>2</sup>*J*) showed regeneration which was normal. It is obvious that little attention was paid to my concluding paragraph, which emphasised the need for caution and accuracy when working with murine muscular dystrophy.

With an air of finality the authors, to whom these criticisms are directed, concede that tissue culture may no longer be a fruitful research tool in this field.

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<sup>1</sup> Hamburgh, M., Peterson, E., Bornstein, M. B., and Kirk, C., *Nature*, **256**, 219–220 (1975).

<sup>2</sup> Gallup, B., and Dubowitz, V., *Nature*, **243**, 287–289 (1973).

<sup>3</sup> Parsons, R., *Nature*, **251**, 621–622 (1974).

HAMBURGH *ET AL.* REPLY—We admit that the reference to foetal age may have been misleading! Timing of foetal age depends on the method of counting: some investigators call fertilisation, as shown by the appearance of the vaginal plug, day 0 whereas others call it day 1. It is always implied that any designation of foetal age is  $\pm 12$  h.

The designation ‘*dy*’ is an acceptable abbreviation for dystrophic and as the text clearly states that “phenotypically dystrophic mice were obtained from matings between tested homozygotes for the dystrophic gene *dy*<sup>2</sup>*J*”, this should have been sufficient to clarify the point.

As for Parsons’ letter<sup>2</sup> confirming our earlier work, he himself states<sup>2</sup> that “my results are essentially similar to those of Paul and of Hamburgh *et al.*”. Although there may be a difference between “results that are essentially similar” and results that confirm, it is, however, so fine, that we admit, it escaped us. Parsons states<sup>4</sup> that little attention was paid to his concluding paragraph<sup>2</sup> which emphasised the need for caution and accuracy when working with murine muscular dystrophy. We are fully familiar with the differential regenerative capacity exhibited by minced muscle obtained from the 129/ReJdy strains and the C57BL/6J *dy*<sup>2</sup>*J* strains in his culture conditions.

Our comment that the tissue culture set up may not be as well suited as originally anticipated merely expresses an experience shared by other investigators that many genetic defects do not express themselves *in vitro*. Parsons, interprets the sense of this comment to mean that “tissue culture may no longer be a fruitful research tool in this field”.

We should like to take this opportunity to mention, however, that whatever the reasons for the different result, between the experiments by Gallup and Dubowitz<sup>2</sup> and our own<sup>1</sup>, they may well be related to differential expressivity of the *dy*<sup>2</sup>*J* gene. Slightly different tissue culture environments, differences in foetal age, sex and other, yet to be identified factors, come to mind. Genes differ both in expressivity and penetrance in different environments, and gene mutations can often be revealed only by proper challenges. We consider Gallup and Dubowitz’s series to be valid and most stimulating experiments.

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<sup>1</sup> Hamburgh, M., Peterson, E., Bornstein, M. K., and Kirk, C., *Nature*, **256**, 219–220 (1975).

<sup>2</sup> Parsons, R., *Nature*, **251**, 621–622 (1974).

<sup>3</sup> Hamburgh, M., Bornstein, M. B., Peterson, E., Masurovsky, E. B., and Kirk, C., *Progr. Brain Res.*, **40**, 497–508 (1973).

<sup>4</sup> Parsons, R., *Nature*, **259**, 158 (1976).

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## Model ages

HUTCHISON *et al.*<sup>1</sup> have reported U–Pb analyses on samples of the meteorite Nakhla which confirm that this meteorite is younger than 3.68 Gyr. In both their title and their discussion there seems to be some apprehension about the ‘validity’ of model ages. Because of the considerable utility of the concept of model ages, we attempt here to clarify this matter.

Consider the number of atoms of a daughter nuclide ( $D$ ) and of its radioactive parent ( $P$ ) which are present in a sample today. Let us assume that a non-radiogenic isotope of the daughter nuclide is also present in the sample (the number of atoms being  $S$ ). For a given reference ratio  $(D/S)_M$ , the model age ( $\tau_M$ ) of the sample is given by:

$$(D/S) = (D/S)_M + (P/S)(\exp(\lambda\tau_M) - 1)$$

$\tau_M$  is the time taken for the  $(D/S)$  ratio of the sample to evolve from the reference to the measured value if the system has remained closed. The trajectory in Fig. 1 leading to the present ratio (point A) must satisfy  $D(\tau)/S(\tau) = (D/S) - (P/S)(\exp\lambda\tau) - 1$ . Point B (where the real trajectory departs from the equation) represents the last time the system was

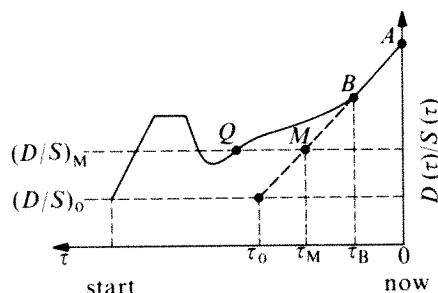


Fig. 1 Schematic diagram showing the evolution of  $D(\tau)/S(\tau)$  with time for an open system. The value  $\tau_0$  yields the maximum age of the sample.

disturbed. The time when the system last passed through  $(D/S)_M$  is at point Q. Estimates of the initial  $(D/S)_M$  for Sr and Pb have been obtained from measurements in ancient samples with low values of  $P/S$  (refs 3–9). Designating these reference values as ‘0’ we have the corresponding model ages  $\tau_0$  given by  $(D/S) = (D/S)_0 + (P/S)(\exp(\lambda\tau_0) - 1)$ . If  $(D/S)_0$  were the lowest possible ratio for solar system material, then the corresponding model age would be a strict upper limit to its age ( $\tau_0 \geq \tau_B$ ) since  $(D/S) - (D/S)_0$  is the largest change for a closed system which is now at  $(D/S)$ . For objects which have remained closed systems since the time of the hypothetical earliest planetary objects,  $\tau_0$  would be the ‘age of the Solar System’. It follows that samples which yield model ages  $\tau_0$  in the neighbourhood

of 4.6 Gyr may represent ancient materials with a relatively simple history. Values of  $\tau_0$  which are much greater or much less than  $\sim 4.6$  Gyr constitute unequivocal evidence of open system behaviour at times less than 4.6 Gyr ago.

In general, the model age  $\tau_0$  must always be greater than the most recent time that chemical differentiation took place. For example, a devitrified glass from Nakhla yields a model age relative to ALL or BABI of  $\tau_{ALL} \approx \tau_{BABI} = 1.78$  Gyr (ref. 2). It is thus evident that Nakhla has been subject to open system behaviour for Rb and Sr, at least on a microscopic scale, more recently than  $\sim 1.78$  Gyr ago. The actual time of the last recrystallisation event was determined by a Rb–Sr internal isochron which gives an age of  $\sim 1.37$  Gyr (ref. 2). In addition to the age of crystallisation or recrystallisation of Nakhla, we can inquire whether this event consisted of local element migration (for example) on a mm scale, of an  $\sim 4.6$  Gyr-old object, or whether Nakhla formed by element mobilisation on a much coarser scale at times much younger than 4.6 Gyr. The Rb–Sr model ages for ‘total’ chips of Nakhla obtained from different meteorite fragments yield  $\tau_{BABI}$  from 3.58–2.97 Gyr, and show that the ‘total’ meteorite was produced by differentiation at times strictly younger than 3.58 Gyr. The model age arguments given above are of course applicable to all parent–daughter systems. The data of Hutchison *et al.*<sup>1</sup> show that ‘total’ rock U–Pb model ages range from 4.10–3.68 Gyr, thus requiring a differentiation time younger than 3.68 Gyr. It is evident that all these data confirm a young differentiation age for the Nakhla parent body.

Based on petrographic features, it is most plausible to consider Nakhla as an igneous rock with a simple history. The Rb–Sr data prove that this object was subject to almost complete isotopic equilibrium 1.37 Gyr ago, and analyses of ‘total’ rocks show that the parent body was differentiated at least once at a time less than 3.6 Gyr ago. While the data define a rather good linear array, however, the precise age of crystallisation or recrystallisation is not exactly defined. The ‘total’ rocks do not lie on the isochron. Since it is reasonable to assume that the total rocks are comprised of their constituent mineral phases, it follows that some mineral phases in the rock must deviate even more widely from the linear array. The small but distinct deviations of the data from an isochron seem to require that the original differentiation which produced Nakhla occurred more than 1.37 Gyr ago, or that the deviations are the result of recent alterations or analytical difficulties.

In the preceding comments we have assumed that the initial values  $(D/S)_0$  were well defined. This has been considered doubtful by Hutchison *et al.*<sup>1</sup>. For the case of Sr, an extensive search for

primitive  $^{87}\text{Sr}/^{86}\text{Sr}$  has been carried out on meteorites and lunar and terrestrial samples<sup>3,4,9,10</sup>. Since the Rb/Sr ratio in the solar nebula is  $\sim 0.5$ , the rate of evolution of  $^{87}\text{Sr}/^{86}\text{Sr}$  corresponds to a change of 0.3% per 100 Myr. It is thus possible that a wide range in initial  $^{87}\text{Sr}/^{86}\text{Sr}$  could be found in ancient Solar System objects if condensation and fractionation processes occurred for a time interval of  $\sim 10^8$  yr. The lowest  $^{87}\text{Sr}/^{86}\text{Sr}$  value so far observed is, however, below BABI by only three parts in  $10^4$  which will not significantly alter the model ages for Nakhla. With regard to the U–Pb system, the basic data as first reported by Patterson *et al.*<sup>5</sup> have been modified over the years but the essential results are not changed<sup>6–8</sup>. If we consider  $^{238}\text{U}/^{204}\text{Pb} \sim 0.3$  in carbonaceous meteorites<sup>7,8,11–13</sup> as representative of the solar nebula, then we calculate a change in  $^{207}\text{Pb}/^{204}\text{Pb}$  at 4.6 Gyr of 0.2% per 100 Myr. This rate is similar to that for  $^{87}\text{Sr}/^{86}\text{Sr}$ ; however, in contrast to the case of Sr, the rate of evolution of Pb in the solar nebula is much less than that on the Earth, Moon and many meteorites, reflecting strong U/Pb enrichment for terrestrial-type planets in the early Solar System. It is possible that the iron meteorites studied for initial Pb were produced by planetary differentiation of a body with high  $^{238}\text{U}/^{204}\text{Pb}$  and could reflect rapidly evolving Pb compositions. The general agreement of troilite Pb with that found in carbonaceous chondrites, which are certainly not planetary differentiates, would, however, seem to exclude the possibility of the large shifts in the value of primordial Pb referred to by Hutchinson *et al.*<sup>1</sup>.

We conclude that model ages provide an upper limit to the age of any system and that total rock model ages provide an insight into the differentiation processes active in planetary evolution. For Nakhla, we conclude that all methods confirm a young formation age for this meteorite, that some complexities are manifest in the detailed history of this object and finally, that no information about primitive initial isotopic compositions can be deduced from data on this young body.

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HUTCHISON, GALE, AND ARDEN REPLY—  
 We are grateful to Wasserburg and Papanastassiou for their courteous and clear discussion<sup>1</sup> of model 'ages' arising from our paper<sup>2</sup> on some preliminary U-Pb data for the achondritic meteorite Nakhla.

We agree that a single-stage model 'age' computed from a primitive Solar System daughter/stable nuclide ratio ( $D/S$ )<sub>0</sub> defines the oldest limit for the most recent time at which the particular system became closed. This is stated in our paper on the Rb-Sr chronology of Nakhla<sup>3</sup>. We agree also that it is likely that model 'ages' greatly different from 4.6 Gyr constitute evidence for multiple stage open system evolution. This has already been stated by one of us<sup>4</sup>. We feel, however, that it is possible that the ordinarily accepted 'primitive' Solar System initial isotopic compositions might not apply to all objects in the Solar System. In our U-Pb studies we have had several indications that they are not universally applicable. Recently discovered anomalous ratios of two light elements<sup>7</sup> suggest that we may still have something to learn about isotopic abundances in the early Solar System.

It is still our view that model 'ages' should not be used without a clear statement of their meaning, as they are readily confused with true ages by those who are unfamiliar with the field of geochronology. Further, when only a limited number of model ages are available, the upper limit set may be so far from the true age as to be misleading. An example is provided by our own Rb-Sr work on Nakhla<sup>3</sup>, where 15 Rb-Sr determinations on diopside, plagioclase, olivine and various magnetically separated mixtures, yield  $\tau_{\text{BAB}}$  model ages ranging from 5.9 to 2.33 Gyr, whereas the internal isochron yields a true age of 1.24 Gyr.

Our data indicate that the Nakhla meteorite crystallised from a melt precisely  $1.24 \pm 0.01$  Gyr ago<sup>3</sup> (not 1.37 Gyr ago<sup>8</sup>) with complete isotopic mixing. Because the U-Pb data demand a history of three (or more) stages (unless unusually low initial Pb isotope ratios are invoked) it is almost certain that a large scale chemical fractionation occurred at that time. This age is, of course, less than the whole meteorite model 'ages'. We reiterate that such a fractionation would have obliterated evidence of the earlier history

of the parent body of Nakhla. 'Gross differentiation' of the parent body (ref. 5, abstract) could, therefore, have occurred before 3.6 Gyr, if one assumes that the later, 1.24-Gyr, event was due to volcanism or impact melting on a less than planetary scale, as is discussed in more detail in ref. 3. Wasserburg and Papanastassiou<sup>1</sup> correctly argue that Rb-Sr and U-Pb model 'ages' for "total" meteorite chips require a differentiation age for the total meteorite strictly younger than 3.58 or 3.68 Gyr respectively (in fact this time is 1.24 Gyr), but incorrectly assume that this argument may certainly be extended to the parent body of Nakhla as a whole.

The presence in Nakhla of water-bearing silicate has now been confirmed, but in addition, evidence of mild, post-crystallisation shock was found<sup>6</sup>. Presumably it was during this mild shock event that the Rb-Sr system was disturbed on a millimetre scale, as shown by the failure of meteorite chip data to lie on the mineral isochron. There is no longer a need to invoke either redistribution of Rb during or after atmospheric flight<sup>3</sup>, or incomplete homogenisation of Sr 1.37 Gyr ago during dry metamorphism<sup>5</sup>. We are pleased that Wasserburg and Papanastassiou now prefer to agree with us in interpreting Nakhla as an "igneous rock with a simple history".

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## North Sea phytoplankton

REID<sup>1</sup> described large scale changes in North Sea phytoplankton communities which have been detected since the mid-1960s by the Continuous Plankton Recorder survey. These changes were characterised by a marked decline in diatom populations, a possible increase in microflagellate abundance, and a decline in the zooplankton biomass. Reid concluded that eutrophication of North Sea waters and climatic changes could not, by themselves, easily explain these alterations in plankton communities.

I suggest that another set of factors, namely the presence of persistent industrial pollutants in the North Sea<sup>2</sup>,

may partially account for these changes. Many species of marine phytoplankton, particularly diatom species, show sensitivity to low levels of industrial waste products, including heavy metals<sup>3</sup>, petroleum hydrocarbons<sup>4</sup>, and stable chlorinated hydrocarbons<sup>5</sup> such as polychlorinated biphenyls<sup>6</sup>. Alterations in the species composition of phytoplankton communities through selective toxicity of pollutants have been observed in the laboratory<sup>5</sup> and in freshwater ponds<sup>6</sup>. The predictions, based on laboratory research, suggesting that pollution-linked alterations in the species composition of phytoplankton communities would result in disturbances of zooplankton communities<sup>5</sup> (due to selective herbivory) are consistent with the observations described by Reid<sup>1</sup>. The degree to which the North Sea plankton are exposed to industrial wastes is subject to further study, though plankton in neighbouring waters are highly contaminated with organic pollutants<sup>7,8</sup>. (It is interesting that sublethal concentrations of pollutants can interact with various environmental factors to enhance pollutant toxicity to marine organisms, including phytoplankton.) Furthermore, North Sea animals higher in the food chain are heavily contaminated with industrial waste products<sup>9</sup>, indicating their general presence in the resident biota.

Longhurst *et al.*<sup>10</sup>, citing the natural variability of marine populations, commented that changes in oceanic plankton communities often cannot clearly be traced to marine pollution. Indeed, it is difficult to pinpoint any single environmental factor as the sole cause of community disruptions, because so many variables may interact to affect biological systems. I propose that the presence of persistent pollutants be considered one of the possible contributing factors influencing the plankton communities in the North Sea.

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<sup>4</sup> Mironov, O. G., *ibid.*, 222-224.  
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# reviews

THIS book\* gives an account of the famous encounter between Lord Kelvin and the geological establishment over the use of physical arguments to set limits to the age of the Earth. It was an epic struggle which continued for 50 years with great vehemence and some acrimony.

In their thoughts about time the geologists of the middle of the nineteenth century were not much inclined to arithmetic. They had inherited from Hutton and Lyell the idea that the Earth showed "no vestige of a beginning, no prospect of an end". This and the related ideas, that things have always been much as they are now, and that causes at present at work could, given enough time, account for all that has ever happened on earth, were the basis of their science and, in large degree, still are.

Very early in his career Kelvin became interested in the time scale of the Earth. His two principal methods of setting an upper limit were the calculation of the time for which the earth's original heat could supply the outward flow observed at the surface and a similar calculation for the sun. Both calculations required few and very plausible assumptions. Kelvin, and most physicists of the time, had great confidence in the results.

Kelvin's first estimate of 100–400 Myr did not greatly alarm the geologists. They no doubt knew that 100 Myr was a finite time, but it was so large a number that it seemed unnecessary to quarrel with it. Unfortunately, as he grew older, Kelvin grew more parsimonious of time and reduced his estimate to 20–30 Myr with threats of further restriction. This was too much, or rather too little; the few geologists who had disliked the 100 Myr limit were joined by the majority of their colleagues and a classic encounter occurred between two groups with almost no ideas in common. Kelvin had the prestige of physics, of Newton and of the divinely prescribed order of Nature behind him. Clearly he could not be wrong. The geologists had gut feelings that any specified length of time was inadequate, but little pos-

sibility of convincing others. Darwin, for example, estimated the time necessary to excavate the Weald of Kent as 400 Myr, but had no real basis for his statement, which he came greatly to regret.

## Epic struggle with geological establishment



Edward Bullard

The story of the collision of two such disparate groups is admirably told in this book. Besides the main contestants all kinds of fascinating figures flit across the scene, including Lord Salisbury (the Prime Minister), Clarence King (the explorer of Colorado and Utah), T. J. J. See (the presumed author of that once celebrated work, *The Life and Remarkable Discoveries of T. J. J. See*) and even, briefly, Frank Harris (the author provides a reference to his *Life and Loves*). The course of the dispute is

set out in detail and is of great interest to anyone concerned with the development of ideas about the Earth. Here the ideas of stratigraphy, evolution and physiography first met theoretical physics. The interest is, of course, enhanced by the fact that the biologists and geologists were right in saying that 30 Myr was not enough time, even though they had no way of providing convincing numerical estimates; how could one say how many years it took to convert *Eohippus* into a Derby winner?

The debacle induced by the discovery of radioactivity is well described; one of the oddest features is that no one seems to have been much concerned that, for the next 30 years, there was still no convincing account of the source of the Sun's heat.

In spite of the careful account of all these things some readers may feel a little deprived. An historian must study the past in its own terms. He must not ask 'What was Henry VIII's attitude to women's lib?'. This viewpoint has been adopted also by historians of science and, in some degree, is clearly necessary. But should one go the whole way? The author of this book consistently refrains from saying that anything is right or wrong, silly or unjustified. If Kelvin assumes that the Earth was initially at 1,500 °C, the author reports that he said it and that it was a more or less arbitrary choice, but he does not add that it matters very little what figure is taken. On the other hand no one would guess that Kelvin's assumption of the absence of convection within the Earth was crucial and that the attacks on it were well based. The history of science is different from other kinds of history; there is the additional fact that some things are correct and some wrong *sub specie aeternitatis*. The reasons for the beliefs are a function of the surrounding culture, but being right or wrong is not a thing of one age alone. In the study of the motions of the planets Descartes was wrong and Newton was right, and so it will always be. It is part of the function of the historian to say how and why. We and he lose a good deal if he backs away from the question. There were geniuses, inspired guessers, cranks, stick-in-the-muds and rash men in the past, just as there are today, and it is a pity to treat them all alike and leave the reader to decide which is which. □

\**Lord Kelvin and the Age of the Earth*. By Joe D. Burchfield. Pp. xii+260. (Macmillan: London and Basingstoke, July 1975). £10.



## Metallic catalysts

*Structure of Metallic Catalysts.* By J. R. Anderson. Pp. ix+469. (Academic: New York and London, June 1975.) £12.60; \$33.75.

THE technical importance of catalysts is such that an understanding of their structure is of obvious importance, whether from the view-point of the extent of surface accessible to reactants, or of the more intimate details of surface topography, chemistry, or electronic structure which might influence adsorption processes. Also, in the case of supported catalysts, the structure of the substrate can determine the state of dispersion of the metal and perhaps contribute to the overall reaction. This book is the first attempt to bring together information in these areas from the plethora of published literature, and from this aspect alone is a considerable achievement.

Available information on the structure of typical metallic phase catalysts is covered in some detail, particularly that relating to model systems where

surface contamination can be more carefully controlled and which can therefore be used as standards for comparison. There are chapters on supports, massive, and dispersed, metal catalysts, and the structure and properties of small metallic particles. The book concludes with an outline of some appropriate techniques for the physical and chemical investigation of such structures, and there is an appendix which describes the preparation of some examples of typical metallic catalysts.

The state and scope of the subject dictate that the work be largely descriptive and of a review nature. It is, however, well and critically written, acceptably illustrated, and generally readable. It should make an admirable starting point for those entering the fields of catalysis or surface chemistry; the wealth of detail, some of it in useful tabular form, and the large number of quoted references, unfortunately without an author index, will also make it a useful addition to the libraries of any academic or industrial laboratory engaged in similar studies.

G. K. L. Cranstoun

## Opiate receptors

*Opiate Receptor Mechanisms: Neurochemical and Neurophysical Processes in Opiate Drug Action and Addiction.* (Based on a work session of the Neurosciences Research Program.) By Solomon H. Snyder and Steven Matthysse. Pp. 116+vi. (MIT Press: Cambridge, Massachusetts and London, June 1975.) \$8.95.

THIS book is a record of a Work Session of the Neurosciences Research Program sponsored by the Massachusetts Institute of Technology and held in Boston during May 1974. The symposium was an unqualified success because it came at a time when the work of several groups, particularly those of A. Goldstein, L. Terenius, S. H. Snyder and C. B. Pert, and E. J. Simon, had greatly contributed to our knowledge of the nature and distribution of opiate receptors.

When the symposium was planned, various lines of indirect evidence had accumulated which suggested that there were in the central nervous system endogenous ligands that interacted with the opiate receptors. Evidence for the existence of such ligands was presented by L. Terenius, and by J. Hughes and H. W. Kosterlitz. G. W. Pasternak and S. H. Snyder mentioned supporting evidence which was updated in the published version.

These two sections are probably the most topical parts of the book. In the introduction, there is a brief review of the chemistry and action of opiates for readers unfamiliar with the field. The second chapter of the book deals with the biochemical identification of opiate receptors and the third with agonist-antagonist interactions. The fourth considers neural mechanisms and contains a discussion by D. J. Mayer of the interesting experiments on analgesia induced by electrical stimulation of the periaqueductal grey matter and its prevention by the opiate antagonist, naloxone. The fifth chapter deals with biochemical phenomena in opiate action and addiction, the sixth with addiction, the seventh with the discussion review on further research. The final chapter is a stimulating and provocative essay by S. H. Snyder on a model of opiate receptor function with implications for a theory of addiction.

This is a well-produced book presenting expertly and critically the state of our knowledge in an important field. Because the various chapters are not written by the individual authors but by the editors with the help of a small number of participants, the style is uniform and pleasing. It is unavoidable that part of the book has already been overtaken by events in a field in which a considerable number of workers are in constant and vigorous competition with each other.

H. W. Kosterlitz

## Hydro, azo, azoxy

*The Chemistry of the Hydrazo, Azo and Azoxy Groups.* Edited by Saul Patai. Part 1: Pp. xiv+1-598. Part 2: Pp. xiv+599-1,190. (Wiley/Interscience: London and New York, September 1975.) £17 each part; £33 two volume set.

THIS is the sixteenth volume to appear in Professor Saul Patai's monumental series on the chemistry of individual functional groups; there are five volumes to come. There is no detailed consideration of azo dyes on grounds of space, and diazonium salts and diazoalkanes are to be covered in a subsequent volume.

The general style, arrangement and coverage closely parallel those of previous volumes, including the usual articles on structure, thermochemistry, electrochemistry, photochemistry, radiation chemistry, mass spectra, basicity and complexing, of the three groups. There are also interesting accounts of chiroptical properties, directing and activating effects, biological formation and reactions, syntheses and uses of isotopically labelled species, rearrangements (a particularly good and interesting contribution), ionic reactions, and radical reactions. There is a useful chapter on preparative procedures, and also two interesting longer chapters on oxidation and synthetic uses, and reduction and synthetic uses, of the groups. Finally there are more specific, and specialised items on transition metal chemistry of the groups, on conformational analysis of hydrazines, and a long but very interesting chapter on the formation and fragmentation of cyclic azo compounds.

The literature seems in general to have been surveyed to the end of 1973—I could find only fourteen references, out of many hundreds, dated 1974—although this datum line does not seem to be uniform for all articles. The subject index could usefully have been a little more comprehensive, perhaps at the expense of the author index that one would have thought to be relatively less important. The general printing and layout of the reaction schemes is markedly superior to that in most of the previous volumes.

Peter Sykes

**Erratum.** Gordon and Breach, publishers of *Composition of Cosmic Radiation* by Apparao, reviewed jointly with another book on the same subject (*Nature*, 258, November 6, page 40), point out that the reviewer's statement about the comparative prices per page of the two books is incorrect. The book by Apparao has, in fact, the lower price per page. We apologise for the error.

*Interfacial Phenomena in Metals and Alloys.* By Lawrence E. Murr. Pp. xiv+37. (Addison-Wesley: Reading, Massachusetts, and London, September 1975.) Cloth \$24.50; Paper \$14.50.

THE five chapters of this book make up a comprehensive treatment of those aspects of surface science which contribute to the properties of materials, and in particular to metals and alloys. The first is concerned with surface thermodynamics of solid surfaces, starting from Gibbs' model, but including a discussion of the distinction between surface stress and surface tension, and the effect of vacancies and other singularities of structure. In the second chapter these principles are applied to the description of conditions for the establishment of interfacial equilibria, especially the evaluation of interfacial free energy ratios. Because of the crucial importance of interfacial free energy values in determining the forms of crystals and grains, chapter 3 is concerned with their determination in different systems. The last two chapters treat the structure and properties of interfaces respectively.

A particular feature of this book is the wealth of factual information which has been gathered into tables of surface and interfacial free energies of solids, and related quantities, occupying almost 10% of the available space. Taken with over 800 references, this ensures that the book will be extremely useful as a source of critically appraised facts for research workers in addition to its primary function of instructing students of materials science in important principles, and exercising them with fifty problems, mostly numerical. Comprehensive author and subject indexes have been provided, and the book is very clearly printed and copiously illustrated. **A. Couper**

*Organoborane Chemistry* (Organometallic Chemistry: Series of Monographs.) By Thomas Onak. Pp. x+360. (Academic: New York, San Francisco and London, July 1975.) \$38.00; £18.25.

THIS book is one of an important series on organometallic chemistry, edited by P. M. Maitlis, F. G. A. Stone and R. West. The topic of organoboron chemistry has developed very rapidly: this is the first review for almost ten years dealing explicitly with compounds having boron-carbon bonds, although others are available

on related topics, such as hydroboration. Excluded are other organic boron compounds such as boron esters.

A brief introduction (1 page) is followed by Chapter 2 (18 pages), concerned mainly with X-ray, spectroscopic, and thermodynamic data. Chapter 3 (116 pages) deals with methods of B-C bond formation and cleavage. [For those familiar with the field it will be clear that the author has used a very similar presentation to that used in my article in Muetterties' monograph of 1967.] Chapters 4-6 (80 pages), on four-coordinate organoboranes, organodiboranes, and other polyboranes, are particularly welcome.

## Books brief

There are nearly two thousand references which include papers published in 1973. Inevitably, therefore, the discussion is selective, particularly as about a quarter of the 224 pages of text is taken up by tables. Nevertheless, the monograph is informative and well-written and should be essential reading for anyone active in this field. **M. F. Lappert**

*Interactions on Metal Surfaces.* (Topics in Applied Physics, Volume 4.) Edited by R. Gomer. Pp. xi+310. (Springer: Berlin and New York, 1975.) DM78; \$33.60.

THIS book contains seven articles on selected aspects of the chemisorption of gases on metals. A model (chapter 1) consisting of an alternating potential within the metal and a discontinuity at the surface enables the calculation of wave functions near the surface using generalised Wannier functions. The electron work function is then calculated from a continuum model into which the effect of crystallinity is introduced as a perturbation, and adsorption as localised screening. The chemisorption bond (chapter 2) is described initially in terms of an LCAO-MO model, which is extended to include overlap between metal and adsorbate states and lateral interactions.

Four chapters then give an account of experimental aspects of chemisorption, the first summarising studies of

clean surfaces and adsorption using a variety of techniques, whereas the other three discuss thermal and electron impact desorption, photoemission and field emission spectroscopy, and low energy electron diffraction. The final chapter on heterogeneous catalysis is included to indicate, in the editor's words, "that there is more to interactions on surfaces than the adsorption of hydrogen on the (100) plane of tungsten".

There is some overlap in subject matter between the chapters, but each presents its author's individual view of recent advances. There are over 700 references, a useful index and many excellent illustrations. It is clearly not a 'first text-book' of chemisorption, but research workers will find it both stimulating and informative. **A. Couper**

*Genetics and Psychopharmacology.* (Modern Problems of Pharmacopsychiatry, Volume 10.) Edited by J. Mendelewicz. Pp. viii+132. (Karger: Basel, London and New York, 1975.) Sfr.58; DM55; \$24.25.

PSYCHIATRY relies on a wide range of investigational techniques ranging from the psychoanalytical to the biological. Among the latter, genetic studies have suggested that many major psychiatric illnesses have a hereditary component. The introduction of effective drugs for these illnesses has emphasised the importance of biological factors in the causation and mechanism of psychiatric illnesses. In this book, the two threads are drawn together. The points of contact, however, remain few and one or two of the contributions in this multi-author book contain little or no genetics. The chapters are of a generally high standard and range widely from the role of heredity in alcoholism to the genetic mechanisms governing the rate of acetylation of the monoamine oxidase inhibitor, phenelzine. Monoamine oxidase is the main interest of almost half the book but claims that levels of this enzyme in blood platelets are abnormal in patients with depression and schizophrenia and in relatives of the latter seem premature. Brain levels (surely more relevant) are quite normal in psychiatric patients. One is left wondering whether the greatest difficulties in biological psychiatry relate not to the biological techniques but to the unsystematic way in which diagnoses are made and symptoms assessed. **M. H. Lader**



# obituary

The malariologist **Meir Yoeli** died suddenly on December 5, 1975 in New York. He was born in Lithuania in 1912 and graduated with an M.Sc. from the University of Kaunas in 1934. Soon afterwards, he fled to Palestine and was appointed a lecturer at the Hebrew University of Jerusalem in the department of tropical medicine. Here he was introduced to the subject of malaria by two great masters—Gideon Mer and Saul Adler F.R.S., and continued this work in 1938, in a year's study with Missiroli and Angelini in Italy. Yoeli received his MD at Basel in 1939, and served under Colonel George Macdonald as a malariologist with the R.A.M.C. in World War II. Some years after the war, he left Israel for the United States of America, where he obtained an appointment in the Department of Preventive Medicine of the University of New York, and where he continued to work until his death.

Yoeli is best known for his research in rodent malaria. He had set his heart on solving the problem

of the life cycle of these parasites—a problem which had defeated many other investigators. He first succeeded in transmitting *Plasmodium berghei* through the mosquito in 1951 at the London School of Hygiene and Tropical Medicine, but it was another 13 years before he discovered the clue for producing viable infective forms (sporozoites): the secret of success lay in dropping to 19 °C, the ambient temperature in which his infected mosquitoes were kept—the same temperature at which natural transmission occurs in the cool highland forests of Zaire. This unexpected result was followed by another, equally spectacular—the rapidity of the exo-erythrocytic cycle of the sporozoite in the rodent's liver (2 d instead of the usual 8 d in primate malaria). Much of this work was done in collaboration with his Belgian friend—the late Professor Ignace Vincke—who was the original discoverer of rodent malaria in 1948. Yoeli extended his research to the immunological aspects of the infection, and recently made important contri-

butions to the aetiology of leukaemia.

Yoeli was as interested in art as he was in science. In 1974 he became an honorary member of the Hellenic society for the History of Medicine, and only his premature death prevented a compilation of his Hebrew poetry, literature and philosophy for publication. He will be greatly missed by his family and his friends all over the World.

**P. C. G. Garnham**

**Dr James Linzell** died on December 28, at the age of 54. After qualifying and working as a vet, he obtained his Ph.D. at Edinburgh in 1951. He spent the rest of his career in the Institute of Animal Physiology in Cambridge, where he became the Head of the Department of Physiology.

His major interest was the physiology of the mammary glands of farm animals, and his discoveries about the mechanisms for the starting of, supply to and production by these organs underpin the present understanding of the subject.

## announcements

### International meetings

March 1–4, **Breast feeding and the mother**, London, The CIBA Foundation, 41 Portland Place, London W1N 4BN, UK).

March 1–5, **Tunnelling '76**, London (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1N 4BR, UK).

March 1–5, **Oilseed and vegetable oil processing technology**, Amsterdam (World Conference, AOCS, 508 S. Sixth Street, Champaign, Illinois, 61820).

March 1–5, **Analytical chemistry and applied spectroscopy**, Cleveland (Dan P. Manka, Program Chairman 1976, Jones and Laughlin Steel Corporation, Graham Research Laboratory, 900 Agnew Road, Pittsburgh, Pennsylvania 15230).

March 2, **Applications of linear free energy relationships in biological systems**, London (The Assistant Secretary, Society of Chemical Industry, 14 Belgrave Square, London SW1X 8PS).

March 2–3, **The working diver**, Colum-

bus, Ohio (Debra Klamforth, Battelle, Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201).

March 9–13, **Physics in industry**, Dublin (Eon O'Mongain, Organising Secretary, Physics Department, University College, Dublin, Ireland).

March 15–19, **Third international conference on culture collections**, Bombay (Professor F. Fernandes, Chairman, Local Organising Committee, Bombay University Club House, B. Road, Churchgate Reclamation, Bombay 400020, India).

March 17–18, **The semi-arid areas of the world**, London (The Executive Secretary, The Royal Society, 16 Carlton House Terrace, London SW1Y 5A9, UK).

March 22–23, **Information for the water industry**, Reading, UK (The Conference Organiser, Water Research Centre, Medmenham Laboratory, PO Box 16, Ferry Lane, Medmenham, Marlow, Bucks SL7 2HD, UK).

March 22–23, **Genetic engineering**, Glasgow (Dr P. J. Goddard, Organising Secretary, Nucleotide Group

Meeting, Department of Biochemistry, University of Glasgow, Glasgow G12 800, UK).

March 23–26, **Fundamental and applied dosimetry**, Saclay, France (M. Y. Le Gallic, Secrétaire général du VIII<sup>ème</sup> Congrès International de la S.F.R.P., C.E.N. Saclay, B.P. n° 2,91190, Gif sur Yvette, France).

March 25–26, **The controversy about sweeteners**, Washington, D.C. (Barbara Jorgensen, NAS Office of Information, 2101 Constitution Avenue, N.W., Washington, D.C. 20418).

March 26, **Fish farming**, Edinburgh (The Executive Secretary, The Royal Society of Edinburgh, UK).

March 28–April 1, **International symposium on fluorescein angiography**, Ghent (Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, The Netherlands).

March 29–April 1, **International symposium on urinary stone formation**, Davos, Switzerland (Professor H. Fleisch, Department of Pathophysiology, Murtenstrasse 35, CH- 3008 Bern, Switzerland).



January 22, 1976

## Negotiate flexibly, but explain publicly

THE Royal Commission on Environmental Pollution, chaired by Sir Brian Flowers, has just reported on air pollution control (Cmnd 6371, HMSO, £1.75) and manages to handle the potentially inflammatory issue of the Alkali Inspectorate in a sensible and pragmatic way. The inspectorate is praised and criticised in about equal amounts, but no major modifications are proposed to its traditional mode of working, namely by collaboration with industry rather than by confrontation.

Air pollution control in Britain is carried out through two very separate administrative machineries. Her Majesty's Alkali and Clean Air Inspectorate is charged with overseeing a limited number of specified processes deemed to be particularly polluting or problematical. (The first of these processes was initially associated with alkali works where, from 1820 on, great clouds of hydrochloric acid gas were released in the manufacture of sodium carbonate from salt—hence the quaint name given to the inspectorate when it was established in 1863.) Inspectors have always worked on the principle that industry should use the “best practicable means” to reduce discharges rather than be held to fixed emission standards. In the course of this, the inspector has played a substantial and unsung role in the rapid transfer of information about anti-pollution technology, and has also been consulted as a matter of course before the installation of new plant. But in spite of continued advances in freeing the air of industrial pollutants, the inspectorate has come in for plenty of criticism. The “best practicable means” approach, its critics allege, can hide all sorts of abuses or agreements to go easy on polluters. The inspectorate has compounded the problem by making inadequate responses to its detractors, partly, no doubt, because inspectors feel themselves constrained by industrial secrecy and partly because staff numbers are only adequate to perform the inspecting job, not to hold colloquia or confrontations with a broader public.

The other means for controlling air pollution is through the local authority, whose environmental health officer must take care of domestic pollution and of those industries not on the inspectorate's list. Some authorities have air pollution units, but many do not, so control is patchy. Relations between the inspectorate and local authorities vary from good to bad (there is no administrative reason why they should ever meet). And for all that some cities have cleaned up the air dramatically over the past 20 years (London now has 70% more sunshine in December than it used to before smokeless zones were introduced), 40% of the nation's premises which were originally planned to be in smoke control areas have still to come under this constraint.

It is the commission's hope that by releasing the inspectorate from the Health and Safety Executive, where it never really belonged, and by subsuming it in a larger Pollution Inspectorate which could deal with other problems such as water pollution and solid waste, the necessary central expertise can be preserved and augmented while making it easier for local authorities to deal with this expertise. This depends on two fairly important assumptions: first, that the major problems in water and solid waste management are amenable to the same sort of negotiation that the inspectorate has evolved for air pollution. There seems no reason why they shouldn't be if a national unit is put together from existing staff in water authorities, central government and so on, but positive results may still be several years off. Second, the Pollution Inspectorate really must take its relations with the public seriously and must hire people appropriately. It cannot simply be allowed to become an expanded Alkali Inspectorate, full of very dedicated experts, employed full time in talking to industry; at least 20% of the staff should be available to talk and listen regularly to the public.

The commission talks of the housewife whose washing has been dirtied by a breakdown in pollution control at a nearby plant and who may be “disconcerted and irritated” by a request to leave a message on the District Alkali Inspector's phone answering machine (inspectors only have part-time secretarial staff!). She might be lucky even to find the right phone number. In the London Phone Directory there is nothing under “Air”, “Alkali”, “Clean Air”, “Clean Air Council” (which advises the government on air pollution), “Her Majesty's Alkali and Clean Air Inspectorate”, or “Pollution”. A very smart housewife might just possibly alight on “Environment, Department of the”, where lurking under a sub-sub-heading is “Noise, Clean Air and Waste”. Or she might try her local authority for which, if the phone book is not too out-of-date there could be an entry “Environmental Health Services”, but no mention of air pollution. But, of course, if it was smoke from a bus she would have to ring London Transport. And what about smoke from a car?

Nowhere is bureaucracy more anonymous than in the phone book. The first job for a new pollution inspectorate might be to follow the example of some other countries, hire some intelligent telephone-persons and put a number in the directory with the entry

AIR POLLUTION from all sources—  
complaints and queries . . .

No charge for this public service . . .

□

# Planning to use science . . .

*Vera Rich looks at the Soviet Union's recently published Five Year Plan, in which the prominence usually accorded to science and technology is again apparent.*

AS EXPECTED, the Soviet Union's Five Plan for the period 1976–1980, soon to be submitted to the party congress, gives top priority to greater efficiency throughout the economy, specifically in agriculture and industry. But the framework of the Plan as a whole considerably curtails the almost rhetorical aims for science also expressed by the Plan—namely, “the further expansion and deepening of investigations of the laws of nature and society, increasing its contribution to the solution of problems of current importance in the construction of the material and technological basis of communism, accelerating scientific and technical progress and the growth of effectiveness of production, increasing the well-being and culture of the nation, and shaping the communist outlook of the workers”.

The Plan's modest targets for growth, investment and production seem to have been set deliberately low to take into account possible set-backs of the sort that hampered the previous Plan, such as crop failures and inefficiency. The target for national income growth, at 24–28%, is lower than the 28% achieved by the 1971–1975 Plan. Industrial production will rise 35–39% as against 43% previously, while the growth of investment in agriculture is to fall from 60% to 30%. Under the general slogan of improved “quality” and increased production, it is demanded that 90% of industrial growth and all the growth in agriculture and production is to be met by higher labour productivity (as opposed to 80% in the previous plan).

Within this general prospect of harder and better work with no encouragement of major initiatives, which is seen by many as the design of ageing politicians whose active lifetime is unlikely to run to the full term of the Plan, the scientific projects selected for specific mention and encouragement form an interesting cross-section of research. At one end the emphasis on pure and applied mathematics is related in the Plan to the need for the more efficient and extensive computerisation of the economy—growth in the computer and automated equipment sections, at 60–80%, far outstrips the anticipated average for the industrial sector as a whole, and there are calls for cuts in the share of manual labour in production

At the other end there is the use of space research for the study of natural resources, meteorology and navigation, and for “other needs of the national economy”. Nuclear, plasma, solid state and low temperature physics also receive special mention, as do quantum electronics, optics and astronomy; the need for new materials is stressed, especially magnetic semiconductors, superconductors, and “technically valuable” crystals, and the whole physics programme is grouped in the Plan with the need to develop atomic power and other new sources of energy. Indeed, energy-selected industries, including the chemical and petrochemical sectors, will be among the fastest-growing. The 1980 oil target is up 27%, and a rise of 42% in natural gas will enhance the country's potential as an energy exporter.

In a similar manner, the biology programme is linked both to the need to develop new crops and improvements in occupational health. The main aims for agriculture include the development of animal husbandry and the fodder base, but there will be strong pressures for an improvement in agricultural productivity.

To a certain extent, such elaborations of the purposes of research projects in a time of financial cut-backs may be considered as a justification for their continuation. But the problem with Soviet science, which for all the latitude its practitioners possess operates within a rigid system of state planning, is more complex. With only limited resources of labour and capital available, certain projects—the space programme, for example—acquire a favoured position over others as a result of a complex decision-making process in which potential defence applications or international prestige can be important.

Apart from enjoying considerable material benefits, scientists working in these areas usually find few obstructions in the pursuit of their work. It is possible, for example, to order equipment from abroad; and the procedures for obtaining experimental materials are considerably facilitated. As a result, these fields inevitably attract a great majority of the most gifted talent. On the other hand, scientists not working in a “favoured” field find themselves at a considerable disadvantage, at least with respect to the bureaucracy: requisitions for experimental materials

must be made far in advance and often can only be fulfilled by an unofficial exchange of surpluses between laboratories or institutions.

The gap between favoured and non-favoured projects and institutes tends to widen as a result, and once a particular subject, say space research, has acquired a measure of prestige, a major cut-back is more difficult to order, at least in the political life-time of those who first sanctioned it. As for the non-favoured projects, these tend to be staffed by the relatively less able, resulting in a relative decline in performance, so that in certain fields, like precision instruments, a technological short-fall emerges which makes the purchase of required equipment abroad more likely.

Accordingly, although throughout the Soviet Union's history the presumption has been that Soviet science must inevitably outstrip that of the west, the day of total independence of Soviet technology still lies in the future. This is tacitly admitted by the present plan, which includes the purchase from the west of equipment for producing mineral fertilizers and for the development of the oil, gas, paper, cellulose, and “certain other” industries. The stress on the need for computerisation and automation suggests that there will be considerable (if unacknowledged) reliance on western know-how.

One possible solution open to the authorities is the purchase of foreign equipment which can then be studied and reproduced, thus saving research and development costs. The Plan's proposals may therefore be a disguised way of purchasing expertise. During the last few years the Soviet Union has signed a number of agreements on technological cooperation with Western countries which are clearly intended to be of bilateral benefit, and it will be interesting to observe how the fields of cooperation may change in the future as the Soviet Union closes the short-fall gap in this or that branch of technology.

The sensitive relationship between science and industry in the Soviet Union will continue to be revealed over the coming five years. In the short term, because the rigid targets imposed on industry often leave no time for re-tooling or re-equipping, the introduction of some cost-saving and efficient new process may be reflected in the quarterly returns as a falling-off in various industries. The contribution of science to the long term aims of higher productivity and improved efficiency, to be effective, will demand better management and planning of techniques. Yet, oddly, this is a comparatively neglected area in the new Five Year Plan. □

## ... in public or private?

*Colin Norman in Washington examines the arguments which two recent events involving Soviet dissident scientists have helped to regenerate in the USA's scientific community.*

ON DECEMBER 12 last year, Sergei A. Kovalev, a Soviet biologist, was convicted of "anti-soviet agitation and propaganda" and sentenced to seven years in prison with hard labour, to be followed by three years of exile. His offence was that he had spoken out in defence of human rights and disseminated outlawed literature. Although he had been in jail for nearly a year waiting trial, Kovalev's plight raised little outcry in the West.

In contrast, early this month Leonid I. Plyushch, a mathematician, was released from a psychiatric institution in which he had been forcibly detained for nearly three years, and allowed to leave the Soviet Union. Plyushch's incarceration, for offences similar to those charged to Kovalev, had provoked many public protests from groups in the West, including scientific organisations and the French Communist Party.

To many observers here, those two events underline a simple and obvious fact—public outcry in the West can play a decisive role in tempering the Soviet government's repression of so-called dissident scientists and intellectuals. And that is precisely why the National Academy of Sciences, the most prestigious scientific organisation in the United States, is being pressured to make more public representations on behalf of individual scientists in the Soviet Union, and to speak out more forcibly in defence of human rights.

Although a few individuals have long urged the Academy to adopt a more aggressive public stand on such matters, the issue has recently received some publicity because of an open dispute between the Academy President, Philip Handler, and Jeremy J. Stone, Director of the Federation of American Scientists (FAS), a liberal organisation with a membership of 7,000 which lobbies for such causes as arms control.

The dispute centres on a brief item in the December issue of the FAS newsletter, in which Stone related three complaints about the Academy's public posture in regard to Soviet dissidents, which he heard directly from beleaguered Soviet scientists. Stone picked up the complaints during a recent visit to the Soviet Union on behalf of the FAS to investigate the problems faced by Soviet scientists who have criticised official policies or who

have applied for exit visas; he printed the complaints as part of a detailed account of his findings.

Handler was incensed by Stone's reporting of the complaints because he claims that it represents a gross distortion of the Academy's actions. He fired off an angry, eight-page letter to the FAS president, Philip Morrison, defending the Academy's record, accusing Stone of an "ugly act", and demanding an apology. Beneath the anger, however, lies a serious issue, namely, what is the best way in which the Academy can use its prestige to seek relief for harassed Soviet scientists, or for scientists in similar predicaments elsewhere in the world?

The Academy operates a number of scientific exchange agreements between the United States and other countries, a fact which gives Academy officials extensive contact with their counterparts in the Soviet Academy of Sciences. In the past few years, those contacts have been used occasionally for private, face-to-face representations by the Academy to seek relief for individual Soviet scientists who have been harassed, dismissed from their jobs or imprisoned for such offences as applying for visas to emigrate to Israel, criticising Soviet policies or speaking up in defence of human rights.

In 1972, for example, during a visit to Washington by M. V. Keldysh, then President of the Soviet Academy of Sciences, Handler and other Academy officials took the opportunity to protest the imposition of hefty exit taxes on scientists who had applied for emigration visas. And early in 1973, during a visit to Moscow, Handler made repre-

sentations on behalf of Benjamin Levich, an eminent Soviet electrochemist who had been fired from his job after applying for a visa to emigrate to Israel. Handler says he took up Levich's case with Keldysh and President Podgorny, and he even asked Keldysh to deliver a letter to Levich awarding him a prize from the American Electrochemical Society and inviting him to the United States to give a lecture. Keldysh refused to accept the letter and accused Handler and the Electrochemical Society of "playing politics".

Handler was therefore particularly incensed by one of the complaints in the FAS newsletter, that during his visit to Moscow he had snubbed Levich by refusing to meet him. Handler said that Levich in fact telephoned him at his hotel an hour after he arrived and invited him to his house. Handler says he refused because such a visit would blunt the impact of his private negotiations on Levich's behalf, a position which is consistent with his view that the Academy should conduct such negotiations in private, without making a public fuss. Similar sentiments are expressed by George S. Hammond, the Academy's Foreign Secretary. Hammond says that "having chosen the quiet diplomacy approach, I think we should stick to it. If we go to Moscow and hold private discussions and then make public statements, it would be a breach of confidence and undermine our position".

Stone, however, takes a different view. "All of my Soviet experience during six visits," he says, "suggests that complaints made in private are often ignored, while those made publicly must be dealt with." As far as Handler's refusal to visit Levich is concerned, Stone notes that because of the publicity which would have attended such a visit, "I have no doubt that Levich was more interested in having Handler visit him while he was in Moscow than in any representations that Handler could have made for him privately".

The Academy has, however, made one very public protest. In September 1973, when the press campaign against Andrei Sakharov was at its height, Handler sent a sharply worded protest to Keldysh, warning that unless harassment of Sakharov ceased, scientific co-operation between the United States and the Soviet Union could be jeopardised. Shortly thereafter, the press campaign abruptly stopped. Though Sakharov has, of course, come in for more criticism and intimidation since, it is widely believed that he is no longer in imminent personal danger. According to Pavel Litvinov, a Soviet physicist who was exiled to Siberia after protesting against the Russian invasion of



Jeremy Stone, FAS Director



Czechoslovakia, and who is now living in the United States, "the representation by the National Academy of Sciences in the case of Sakharov was effective. Of that I am sure". He added that "in every case when the Soviet bureaucracy has given in, it has been done by open pressure". Handler maintains that the public protest on Sakharov's behalf was made in response to a critical situation—the fear that Sakharov was about to be arrested and tried for treason. He suggested that such an approach should not be used in less dramatic situations.

Stone believes, however, that the initial success of the Sakharov protest should convince the Academy that occasional public protests would greatly strengthen its hand in private negotiations. And Lipman Bers, an Academy member and President of the American Mathematical Association who has made representations on behalf of beleaguered scientists in several countries, notes that "my impression and experience is that so-called quiet diplomacy and public protests reinforce each other". Similarly, Harrison Brown, a former Foreign Secretary of the National Academy of Sciences who played a key role in the Academy's protest over Sakharov, suggests that "if anything, the Academy has erred on the side of not doing enough publicly", though he adds that he believes that the private approach "has tempered Soviet actions" and notes that "it is very difficult to get a proper balance between public and private approaches".

Stone, meanwhile, is working to get the prestige of Academy members behind some public protests to be launched by the FAS on behalf of dissident Soviet scientists. Last month, he sent a letter to every member of the Academy, asking whether they would be willing to lend their support to petitions "for scientists being denied the right to function as scientists". He said last week that he anticipates a positive response from about 25% of the Academy's members. In addition, he has circulated a petition among physicist members of the National Academy of Sciences asking for their support for Andrei Tverdokhlebov, a physicist who was arrested nine months ago for allegedly disseminating false material and whose trial is imminent. A very high proportion has already responded. A petition has also been mailed to some 20,000 biologists in support of Kovalev. Stone also attempted, unsuccessfully, to ensure that international observers would be allowed at Kovalev's trial.

The Academy is therefore under some pressure to take a more aggressive, public stand in support of dissident Soviet scientists. It should be noted, however, that such a move would draw

strong criticism from the State Department and other government agencies since it would seem to run counter to the spirit of detente. The Administration would much prefer a quasi-government body like the Academy to work behind the scenes, leaving the public protesting to private organisations like the FAS.

The issue of how learned societies should handle relations with their

counterparts in the Soviet Union has, of course, also been aired in other countries. In the UK scientists debated the subject on television in 1973, and in 1974 the Council of the Royal Society considered the issue following an initiative by Professor John Ziman. The continuing low profile of the society makes it reasonable to assume that proposals for a more public stance were turned down. □

## An appeal for help

THE following quotations are taken from a letter written by Valentin F. Turchin, a Soviet mathematician who was fired from his job in July 1974 after he had made a public statement in defence of Andrei Sakharov. Turchin, who is chairman of the Soviet group of Amnesty International, has been out of work for 18 months, and has applied for permission to visit the United States to work at Columbia University. He was informed on December 15 that his application had been denied. The letter was received on January 13 by Jeremy Stone, the FAS Director. Turchin says that he wants it to be discussed by the scientific community in the West. It will eventually be published in full by the Khronika Press in New York.

Turchin begins by describing the harassment and the trial last month of Sergei Kovalev. Kovalev, an eminent biologist, was given the maximum sentence of 7 years' imprisonment with hard labour and a further three years of exile within the Soviet Union for "anti-Soviet agitation and propaganda". Noting that, together with Sakharov, he had appealed for help for Kovalev from Western scientists, Turchin states that "there was no response deserving to be mentioned and I don't know whether there was any response at all . . . No action was made which could have attracted serious public attention and influenced Soviet authorities. The world scientific community betrayed Kovalev".

He continues: "You are very proud, my dear colleagues, that you separate science from what you call politics. You do not go in for politics, you say. Neither do we. Dissidents in the Soviet Union do not go in for politics: they struggle for air. What you are separating science from is not politics but mere decency. And in fact, it is not separation, but a reversal, changing of the sign. For whatever you think, you are not neutral in the conflict between totalitarianism and freedom. You actively cooperate with totalitarianism, support it . . .

"People of science are intrinsic enemies of totalitarianism, because they professionally need intellectual freedom. The core of the Soviet dissidents consists mainly of scientists. But the state presents to the scientist a dilemma: either to support totalitarianism, to lie and betray comrades, or to challenge it to some extent and pay in proportion, by professional losses up to the point of losing work and freedom. The Western scientific community helps to conduct this policy by fully accepting the totalitarian rules of the game in scientific contacts with the USSR and the satellite countries. One example will suffice: did you ever turn back a Soviet delegation because the scientists you had invited were not included [because they were] politically unreliable? Politically reliable people, that is those who help strangle the recalcitrant, are allowed by the Soviet authorities to come out on the international scene. You give your sanction to this selection . . .

"Why not demand, for example, that a small proportion of those who participate in scientific exchange—say, one in ten—must be the other side's choice, and if not, then firmly refuse to cooperate? Scientists hold powerful levers of influence on totalitarian countries. Why do they not use them to save a colleague from imprisonment? . . .

"The detente is necessary, I'm completely for the detente. But in the absence of strong public pressure for human rights all over the world the detente will automatically lead to proliferation of totalitarianism. The Helsinki agreement reveals a typical pattern: the West makes real concessions in exchange for imaginary ones from the East. After Helsinki, the situation with human rights in the USSR has become only worse . . . The proponents of the Helsinki agreement argued that it would provide the grounds for exerting pressure on the USSR for exchange of people and ideas. But what is the use of the grounds if there is no desire to exert pressure? . . ."

## CANADA

## Royal Society may acquire an expanded role

*The ways in which Canada's Royal Society might have a broadened role in the country's scientific affairs is becoming a matter of some controversy. From Ottawa, David Spurgeon reviews the main issues, which focus chiefly on the question of relations with the government.*

THE contract for \$75,000 over a 12-month period which was signed not long ago between the Canadian federal government and the Academy of Sciences of the Royal Society of Canada will enable the academy to undertake a study to identify specific missions in the natural and human sciences that may be of value to the government, and to recommend policies and procedures for carrying them out. Thus, apart from allowing the academy to engage an executive director half-time—he is Donald Hurst, former head of the Atomic Energy Control Board of Canada, the country's regulatory agency in nuclear affairs—the contract may give it a broadened role in Canada's scientific affairs, the implications of which do not delight all its members.

A number of initiatives designed to make the Royal Society more influential in scientific affairs has been taken in recent years. Five years ago, a committee set up by the Society's old Section Three (now the Academy of Sciences) to study how this could be done became a committee of the Society as a whole within months. Now there are three component academies in the Society, the other two being those of the social sciences and humanities (one for the Anglophone section, the other for the French).

One of the reasons some have felt the need of a stronger role for the Royal Society in science was expressed by the geologist J. Tuzo Wilson when he was president in 1973. In an article in *Science Forum* he said that the Society believed that Canada needed "an active and independent academy, not so much to advise government, but to promulgate informed opinions and to lead open discussions of important issues that will aid governments in making their decisions".

Dr Wilson stressed that, unlike the various bodies the government had established for similar purposes, the Royal Society was not a political body. He suggested that its independence, together with its access to leading scientists in many fields, could provide the alternative in scientific advice that

the country needed. It was his view that the whole direction of Canadian science was in the hands of civil servants and members of councils who are appointed by ministers, and he claimed that the whole direction of science had been "politicised".

Not all Society members have agreed on the role the body should take, however. Although some, like Wilson, thought a detached role (in which the Society would make studies in specific areas) was best, others thought it should be closer to the government and play a more official advisory role.

Part of the Society's expanded role may be in international scientific affairs. In other countries, contacts with international scientific unions are usually made through non-governmental bodies, but in Canada the National Research Council has usually provided the point of contact. Some think this anomalous. For the social sciences, the humanities and engineering, Canada has non-governmental organisations that can act as adhering bodies to the international unions: the Social Science Research Council, the Humanities Research Council, and the Canadian Council of Professional Engineers. But there are no corresponding non-governmental bodies for international contact in the medical and natural sciences.

Some members of the Royal Society believe this is a role the Society should play, as academies do in other countries. But the problem of how this could be done remains because of the large number of professional organisations in different disciplines in Canada and the number of constituent bodies of the international scientific unions. One suggestion is that the Society should play the role of clearing house for the appropriate national professional organisations.

A certain amount of scepticism usually accompanies changes which the Royal Society of Canada makes, mainly because many scientists have become accustomed to thinking of the organisation as a self-congratulatory old boys' club. In spite of this, the 'club' counts among its members many of the country's most distinguished scientists (and lately even a few engineers), and it has been stirring itself in recent years to play a more important public role—for example by organising seminars on such topics as the energy problem. The outcome of this latest move will thus be watched with interest. □

## BRITAIN

## University pay grumbles remain

*Last year British university teachers were involved in 'industrial action' before finally resolving their dispute with the government over salary increases. The government has since introduced its pay policy. This month many academics will receive for the first time the cost of living element of their 1975 salaries settlement. David Walker reports on the discontent that still prevails.*

THE Association of University Teachers (AUT), the university authorities and the British government have agreed that the pay code maximum of £6 a week should be added to all academic salaries under £8,500 a year, and the whole package backdated to October. The final figures for the delayed settlement—the basic element was determined by an arbitration body as long ago as May last year—show that junior lecturers have probably benefited most and now start their teaching careers on nearly £3,200 a year. Some staff, who will no doubt bear in mind Mr Healey's recent warning about "confetti money", have increased their annual salaries by £1,000 in the space of 12 months. And one professor of politics told the world on television before Christmas that he was "aggressively satisfied" with his standard of living.

Under the settlement the starting point for all university academic staff, the foot of the lecturer grade, is £3,174 a year rising through 16 incremental points to £6,446. The scale for senior lecturers and readers will now run from £6,234 to £7,742, and the professorial minimum salary will be just below £7,900. Professors outside the clinical subjects will be on an average of over £9,200, although few of them got the £6 a week element or at least got only enough of it to take them to the £8,500 salaries ceiling. The settlement will run for the duration of the Labour government's incomes policy and Mr Laurie Sapper, the general secretary of the AUT, claims they have extracted from the government a firm promise to revise the scales of payment as soon as economic circumstances permit.

In spite of that, there is a lingering mood of dissatisfaction in those senior common rooms where salaries get discussed—and it is a sign of the times that money now gets talked about in public. Those university teachers who take an active part in their trade union (and a key test of their 'militancy' will come in March when they will vote on whether the AUT should affiliate to

the Trades Union Congress, TUC) are still disgruntled. One of the major issues they still consider unresolved is the question of comparisons between their salaries and those paid to polytechnic lecturers and heads of departments.

Straight comparisons between rewards in the two sectors of British higher education are notoriously difficult to make on account of different opportunities for promotion, the numbers of staff, on relative salary points and the balance in teachers' time between teaching and research. Yet they are made, and the AUT officially considers that many university people are comparatively underpaid.

In addition the bi-annual conference of the AUT last December showed that

the government's handling of negotiations on pay during the past year has left a legacy of bitterness. There is talk of "hatred" for the universities in the Department of Education and Science, discrimination in favour of the polytechnics, and—most disturbing—of a Civil Service plot to do down the universities.

The worst of these suspicions are likely to clear in the coming year, however Mr Fred Mulley, the Secretary for Education, is a genial figure who attracts none of the opprobrium heaped on his predecessor, Mr Reg Prentice. Although the university teachers are likely to vote in favour of joining the TUC, it is unlikely that a moderate body like the AUT will do much to steel its arms for militant action on

the issue of salaries.

But action is promised on two fronts that could have important longer term effects on the structure of the profession. The AUT is planning to press for a revision of the ratios of senior to junior staff, on the grounds that there is a serious promotions log-jam, with a great build-up of those on the upper points of the lecturer and senior lecturer scales with little or no prospect of advancement. On another front the AUT is drawing up new salary claims to be presented to the government on the basis of comparison with the Civil Service. This marks an unwelcome development in the eyes of those who fear that the drawing of such analogies will speed the process of bringing universities into full state control. □

FORTY-EIGHT years ago I published the first of a series of papers on the exponential growth of colonies of duckweed and the circumstances under which exponential growth curves became sigmoid. I was following the fashion at that time. Raymond Pearl was doing elegant mathematical analyses of the growth rates of human populations, and Alfred Lotka's brilliant *Elements of Physical Biology* (surely one of the most seminal books on mathematical biology ever written) came into my hands when I was a third year student, just 50 years ago. It is a book which gave, as long ago as that, a masterly treatment of the conditions for equilibrium in biological systems.

So it is with a sensation of nostalgia that I have followed the publications sponsored by the Club of Rome and supervised by Dennis Meadows. It is touching to watch the rediscovery of exponentials, as though Pearl and Lotka had never lived, and ironical to note how much more credible those writers were, working with a slide rule and a little algebra, than Meadows and his colleagues are, armed with a computer. With these thoughts in mind I turned with hope rather than anticipation to the third volume of studies sponsored by the Club of Rome (*Dynamics of Growth in a Finite World*. Pp. 637; Wright Allen; Cambridge, Massachusetts, 1975). Have the authors, as I hoped, at last recognised the fundamental weakness of computer simulations in which the critical parameters—the adaptation of social values and norms to environmental change—have to be omitted because they cannot be quantified?

If this third volume does nothing else, it illustrates the phenomenon of exponential growth. The first volume weighs 170 g and cost \$2.75; the

second weighs 530 g and cost \$18; the third weighs about 900 g and costs (in sterling) the equivalent of \$37. If one makes the assumptions which are the theme of *The Limits to Growth*, the next volume, due in a couple of years,

## Exponentials again



ERIC ASHBY

will weigh 1½ kg and cost about \$60, and the one after that 5 kg, perhaps.

Of course nobody in his senses makes such assumptions, but then neither does he make similar ones about the exponential increase of pollution in nations which have NEPA or the EEC Declaration on the Environment or the Control of Pollution Act; yet a section in chapter six of this book does make precisely this assumption, and the authors reach a conclusion which I think (without immodesty) I could have reached without a computer: "... as long as there is exponential growth in the generation of pollution, ameliorative measures ultimately do little to prevent the model from exhibiting unacceptable levels of pollution

damage" (page 477).

We owe a debt to Meadows and his colleagues for dispelling the utopian mirage: that material standards of living will continue to go up and up, until Indian peasants enjoy the comforts and amenities now enjoyed by the middle classes in Europe. But they do a disservice to their own cause when they imply that their computer simulations can be used as a guide to policy making. A glance at the present state of the world is enough to demonstrate that this naive approach (even though it does include an element of social feedback through the price mechanism) diverts attention from the much higher priorities in policy making. Long before we are starved of resources or smothered in excess population or choked by pollution we are in for a series of grave geopolitical confrontations, to be resolved either by war or by subjection for nations which need resources to conditions imposed by nations which own resources.

The world's future in the short run depends on how these confrontations are handled. The techniques for handling them are not in the hard sciences (though they will buy time) nor in econometrics (which has a dubious record of predictiveness). They are in social anthropology (to understand the nations which may inherit power), in ethology (to understand our own social behaviour under stress), in political science (to help us to devise political systems to match the complexity of post-industrial society). These are the fields which need massive support for research and development. I think sometimes that Britain suffers from a semantic anomaly: that *Wissenschaft* in Germany and *Nauk* in the USSR cover a much wider spectrum of disciplines than our word *Science*.

# news and views

## Search for organic superconductors

from A. D Yoffe

A THEORETICAL paper published in 1964 by W. A. Little (*Phys. Rev.*, **134A**, 1416) dealt with the possibility of high temperature superconductivity particularly in solids formed from long chain organic molecules. The use of the term "high temperature" is of course a little ambiguous, but a realistic interpretation would put this in the vicinity of liquid nitrogen temperature (77 K) or higher. This theory was based on a particular kind of electron-phonon (vibrational) coupling and it naturally created considerable interest and excitement. First, there were the biological implications and second, there was the vision of a relatively cheap high temperature superconductor for both scientific and industrial use. It should be said that the paper was also greeted with a good deal of criticism and scepticism. This led to a hunt for organic-type solids which would be metals at room temperature and which would remain metals even when cooled to temperatures below that of liquid helium (4 K). This is not an easy problem to solve since pretty well all organic solids are insulators or semiconductors. One exception is of course graphite and the intercalate compounds formed from graphite with, say, alkali metal atoms such as potassium. They are metals in this sense, but they hardly qualify as organic solids.

Crystals of the organic charge transfer complexes such as TTF-TCNQ (tetrathiofulvene - tetracyanoquinodimethane) which are extremely anisotropic in their physical properties, do behave as metals with a conductivity in the region of  $1,000 \text{ ohm}^{-1} \text{ cm}^{-1}$  at room temperature when this is measured along one of the crystal axes. This is the direction along which the molecules are stacked. Further evidence for the metallic nature along this direction came from optical reflectivity experiments using polarised light. When the electric vector of the incident light is parallel to the direction of the stack of molecules (good conductivity direction) then typical metallic free carrier reflectivity is found. When the crystals are cooled below liquid nitrogen temperatures

(round 60 K), however, the electrical conductivity begins to fall, and it seems that the solid changes to a small band gap semiconductor. The solid undergoes what is currently termed a Peierls transition, proposed for one-dimensional metals. In 1973 Heeger and his colleagues at the University of Pennsylvania published some rather startling results on the electrical conductivity for some of their crystals of TTF-TCNQ in which they found the conductivity rose to very high values approaching that for copper, just before the transformation temperature to the semiconducting state. There was talk of superconducting fluctuations and there can be little doubt that these experimental findings created quite a stir and led to the renewed interest in this and similar systems over the past few years.

TTF-TCNQ belongs to the class of solids built up from chain-like structures which are loosely called "one-dimensional conductors". Other examples are  $\text{K}_2\text{Pt}(\text{CN})_4 \cdot \text{Br}_{0.3} \cdot 3\text{H}_2\text{O}$  (commonly abbreviated as KCP(Br)) and the sulphur nitrogen polymer, usually called (SN)<sub>n</sub> polymer. This polymer consists of helical chains of sulphur and nitrogen bound together in the crystal by relatively weak van der Waal's forces. The crystals have the appearance of brass, and in 1975 the IBM group (Greene, Street and Suter, *Phys. Rev. Lett.*, **34**, 577; 1975) reported that crystals of this material did in fact become superconducting, but at a relatively low temperature round 0.3 K. This is the first polymer to be shown to be a superconductor and although the result could be likened to a damp squib when we consider the magnitude of the transition temperature, it is important and provides the hope of synthesising other polymers which have higher superconducting transition temperatures.

Many research groups throughout the world with strong chemical support are at present attempting to synthesise such organic solids which behave as metals at all temperatures and which do not display the unfortunate characteristic of TTF-TCNQ at low temperatures. One interesting development has

been the synthesis of the charge transfer complex HMTSF-TCNQ (hexamethylene tetraselenafulvalinium-tetracyanoquinodimethane) which remains metallic below one degree Kelvin, although it does not become superconducting (Block, *et al.*, *Phys. Rev. Lett.*, **34**, 1561; 1975). Another and rather better example is the organic complex radical anion salt 1,2-di (N-ethyl-4 pyridinium) ethylene<sup>2+</sup> (7,7,8,8 - tetracyanoquinodimethane)<sub>2</sub><sup>-</sup> discussed in this issue of *Nature* (page 201) by Ashwell, Eley and Willis.

A good deal of important physics and chemistry is coming out of this work on solids composed of chains of one kind or another. These crystals are extremely anisotropic in their physical properties, and we find that we are concerned with phenomena such as Peierls distortions, Kohn anomalies, soft phonon modes, superlattice formation, charge density waves and superconductivity. It is clear however that the real goal is the synthesis of organic solids which will become superconducting at reasonable temperatures. The experiments discussed above point the way in which future work might develop. □



### A hundred years ago

THE Ladies' Classes at University College, London, began on Monday last the second term of their eighth session. There was a slight decline in the number of students for the session 1874-75, but the first term of the session 1875-76 showed a considerable advance beyond the highest success hitherto attained. In the Michaelmas term, 1874-75, the whole number of individual students was 199; in the Michaelmas term, 1875-76, just elapsed, the number of individual students was 265. The whole number of tickets taken in Michaelmas term, 1874-75, was 257; in the same term of 1875-76 it was 367. from *Nature*, 13, 235; January 20, 1876



# HeLa takes over

by Sandy Grimwade

ALTHOUGH it was suggested in 1967 by Gartler (*Natn. Cancer Inst. Monogr.*, 26, 167) on the basis of isoenzyme patterns that not all cell lines in common use were what they purported to be, it was not until last year that cytogenetic studies by Nelson-Rees *et al.* (*Science*, 184, 1093; 1974) demonstrated conclusively the extent of contamination. Gartler showed that many commonly used cell lines, supposedly of Caucasian origin, contained the A-type isoenzyme of glucose-6-phosphate dehydrogenase (G6PD), which is in fact found only in approximately 30% of the Negro race. One cell line which legitimately contains G6PD type A is the HeLa line, the oldest and most widely used of all cell cultures, which was established in the early 1950s from the cervical carcinoma of a Negro woman in Baltimore named Henrietta Lacks. The warning attached to that finding was that many cell lines could be contaminated with HeLa.

While identification of a single isoenzyme marker left room for doubt, the advent of chromosome-banding techniques by the trypsin-Giemsa and quinacrine mustard methods showed far more specific markers. Examination of HeLa cell chromosomes by these methods shows four unusual chromosomes formed by fusion of portions of various normal chromosomes. Although there remains some disagreement between various groups of cytogeneticists about exactly which chromosomes have fused to form the markers, there is no divergence concerning the occurrence and appearance of the markers in HeLa cells.

In 1974 Nelson-Rees *et al.* reported that several commonly used cell lines from various human sources contained HeLa marker chromosomes as well as the type A G6PD. A third indication consistent with the claim that these were HeLa cells was that no Y chromosome was present. Several of the cell lines were presumed to be of male origin, and should therefore have shown Y chromosomes, whereas HeLa cells are originally female.

The extent of the HeLa take-over of cell cultures is further documented in this issue of *Nature* (page 211) in a paper from Lavappa, Macy and Shannon from the American Type Culture Collection (ATCC). The ATCC, which preserves cultures of every imaginable microorganism and animal cell, distributes its cell lines to laboratories both in the USA and elsewhere. Several thousand samples of cell cultures are sold on a non-profit basis annually. Shortly after the introduction of the

isoenzyme technique, the screening of the entire stock of human cell lines for type A G6PD was initiated and in the 1972 ATCC Registry of Animal Cell Lines 27 out of 56 lines are flagged as containing this marker isoenzyme. The paper by Lavappa *et al.* presents the first results from a cytogenetic screening programme recently initiated using the newly-available chromosome banding technique. Detailed chromosome analysis of several cell lines reveals that five of the lines, all of which bear the type A G6PD, have HeLa marker chromosomes. Since submission of the paper, several other lines have been tested, and so far all the lines with the suspect G6PD isoenzyme have also been found to bear the HeLa markers. Not all these lines have all four markers, however. Detroit 6 cells, for example, lack HeLa marker (HM) 1, but contain two copies of HM2, 3-4 copies of HM3 and show some variability in HM4. These variations are thought to have arisen by somatic cell hybridisation between the original cell line and contaminating HeLa cells. There is also the remote possibility that these markers have arisen spontaneously in more than one cell line.

The results of the ATCC survey and of several other investigations of cell lines in general use have been combined in an extensive table in a recent *Science* paper by Nelson-Rees and Flandermeyer (*Science*, 191, 96-98; 1976). In addition to the criteria already mentioned, phosphoglucose isomerase and HLA antigen data are included. In all, more than 70 cell lines with HeLa characteristics are listed.

These results should give rise to some reappraisal of results in the many laboratories where these cultures are being used. Investigators who think they are working with liver or bone marrow, for example, may find that they have been dealing with Henrietta Lacks' extraordinary carcinoma all along.

It is interesting to speculate on the reasons for the virulence of HeLa cells and the origins of the widespread contamination. It seems reasonable to say that HeLa cells being the oldest line in existence have been subjected to the maximum selective pressure. HeLa cells have been cultured an untold number of times. Those in the ATCC have probably been passaged more than 400 times since their initial isolation in the relatively unrefined culture conditions of 1951 and are therefore perfectly adapted to laboratory conditions. The chromosome studies at the

ATCC were carried out on cells passaged only once or twice from the frozen seed stock. As the cell lines now shown to be contaminated with HeLa were mostly frozen and deposited at the ATCC in the early 1960s, often after several hundred passages, the contaminations probably occurred long before their deposition at the ATCC in laboratories where more than one cell line was being handled. The basic biochemical or genetic reason for the virulence of the HeLa cells which would account for their ability to take over any cell culture remains a mystery however.

Although several of its human cell lines are now shown to be contaminated with HeLa, and up to half of them are suspect, the ATCC intends to continue carrying these lines in its catalogues. Despite the fact that the cell lines are similar in their chromosomes, they retain individual characteristics, such as growth rate and virus susceptibility, worth preserving. □

## Nova Cygni 1975

from P. J. Andrews

NOVA Cygni 1975, first reported by Honda, was independently discovered by hundreds of astronomers as a bright star in the constellation Cygnus on the evening of August 29. It subsequently became the brightest nova seen since Nova Puppis 1942. First indications that the nova was unusual came when a search for the prenova on the Palomar Sky Survey plates showed no star at the nova's position. The rise in brightness, 18 mag, was greater than any previously known; in fact it led to the suggestion, subsequently refuted, that the object was a supernova.

The most widely held theory for the nova phenomenon is that the prenova is a close binary system consisting of a red dwarf, which is filling its Roche Lobe, spilling matter over towards a white dwarf companion. The period of the binary is shorter for systems containing fainter, cooler red dwarfs. The nova outburst is believed to be due to the flow of material from the red star passing by way of an accretion disk to the white dwarf's atmosphere where it is compressed and heated by the gravitational field until it reaches the ignition temperature for hydrogen burning reactions. Once ignition occurs there is a thermonuclear runaway generating the necessary energy to produce the very hot expanding shell of gas which is seen as the nova eruption.

The development of the nova is the evolution of this expanding region. In general the development is complex with several different systems of absorp-

tion lines present in the spectrum which are due to gas ejection continuing after light maximum. For some novae there are great changes in this outflow which are manifested in dramatic changes in the light curve and spectrum.

Nova Cygni, being bright and easily accessible to all northern observatories, was expected to throw light on the various rival theories for different aspects of the nova phenomenon. Unfortunately it does not seem to be, in any sense, a typical nova.

From comparison of the strengths of the interstellar lines in the early spectrum of the nova with those in the supergiant 55 Cygni, Tomkin, Woodman and Lambert (*Astron. Astrophys.*, in the press) deduced a distance for the nova of somewhat greater than 1 kpc and an intrinsic visual luminosity at maximum of about  $-10$  mag. A similar brightness was obtained from the rate of decline in brightness shortly after maximum by Lindgren and Lindgren (*Nature*, **258**, 501, 1975). This intrinsic luminosity is the highest known for a galactic nova, and the speed of decline is the fastest.

The spectrum during this rapid decline also changes very rapidly and does not show all the 'normal' absorption systems seen in typical novae spectra. Possibly this indicates that for Nova Cygni an instantaneous ejection model is more applicable than the continued ejection models which seem better suited to most novae.

While the decline from maximum light has, compared with many novae, been remarkably smooth, the confirmation (Mancocci, Messi, Natali and Rossi, page 186 of this issue of *Nature*) of a small periodic variation in brightness with a period of some 3 h suggests the possibility that some form of modulation of the nova's brightness is being caused by the orbital motion of the binary.

The observation of coronal lines in the near infrared by Grasdaen and Joyce (see page 187) indicates the presence of a very high temperature ( $\sim 10^6$  K) region where apparently the degree of ionisation is still increasing. They suggest that there is the possibility of determining the physical conditions and abundances in this region which is presumably situated close to the site of the nova explosion. Abundance determination should also be possible for a series of high dispersion spectra obtained at the Royal Greenwich Observatory by Stickland within 8 h of discovery. A preliminary analysis by the author indicates that the strengths of the CII, OII and especially NII lines are greater than would be expected suggesting that these elements have greater than normal abundance. This would agree with the theoretical pre-

dictions of Starrfield, *et al.* (*Astrophys. J.*, **176**, 169; 1972) who indicated that these elements will be enhanced in this way by the nova mechanism discussed above.

Shortly after maximum light the spectrum became dominated by emission lines which became progressively stronger relative to the continuum as the nebular stage was approached. At the same time structure at the top of each emission line became apparent. Thus structure, initially seen as four peaks at each emission line, has subsequently developed so that by the end of September up to eleven peaks were seen and measured in each of several different spectral lines. The derived velocities were constant for each peak, where present, for all the spectral lines measured. The expanding nebula is thus strongly lobed in a very complex manner which together with observations of circular polarisation in H $\alpha$  and H $\beta$  by Kemp and Rudy (*IAU Circ.* 2837) perhaps supports Mustel's ideas that strong magnetic fields are present in novae.

There is, no doubt, an enormous amount of observational data on Nova Cygni accumulating. The first results are only now starting to appear, but Nova Cygni seems so far to have posed more problems than it has solved.  $\square$

## Expression of eukaryotic genes in prokaryotes

from Giorgio Bernardi

MANY of the hopes, and some of the fears, associated with future developments in the field of genetic engineering rely upon the possibility of transcribing and translating eukaryotic genes in prokaryotic systems. It is not yet clear, however, whether the ambitious goal of converting bacteria into factories for the synthesis of medically, agriculturally and industrially important proteins of eukaryotic origin can be achieved without some additional advances in our technology.

Following the pioneering work of Berg and his colleagues at Stanford, restriction endonuclease-generated DNA fragments from various sources have been cloned in *E. coli* by linking them to a plasmid (or a phage) replicon, and then introducing the composite molecules into bacterial cells by transformation. After having been first used to introduce segments of other bacterial plasmids and phage DNA into bacteria (see for example Chang and Cohen, *Proc. natn. Acad. Sci. U.S.A.*,

**71**, 1030; 1974; Hershfield *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3455; 1974; Tanaka and Weisblum, *J. Bact.*, **121**, 354; 1975), this method has been used to clone fragments of eukaryotic DNA, such as *Xenopus laevis* amplified rDNA (Morrow *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1743; 1974), *Drosophila melanogaster* DNA (Wensink *et al.*, *Cell*, **3**, 315; 1974; Thomas *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4579; 1974; Glover *et al.*, *Cell*, **5**, 149; 1975; Tanaka *et al.*, *Biochemistry*, **14**, 2064; 1975) and sea urchin histone genes (Kedes *et al.*, *Nature*, **225**, 535; 1975; Birnstiel *et al.*, *Xth FEBS Meeting*, **38**, 3; 1975).

In order to study the expression of eukaryotic genes in the prokaryotic host, advantage has been taken of the fact that plasmids segregating into minicells (which are devoid of bacterial chromosomal DNA) are capable of plasmid-specific synthesis of functional gene products (Frazer and Curtiss, *J. Bact.*, **115**, 615; 1973; Van Embden and Cohen, *J. Bact.*, **116**, 699; 1973). For both rDNA and histone genes, it has been shown (Morrow *et al.*, *op. cit.*; Kedes *et al.*, *op. cit.*) that transcription takes place in minicells; the fidelity of transcription has not, however, been demonstrated in either case.

A novel approach to this problem has been tried by Chang, Lansman, Clayton and Cohen (*Cell*, **6**, 231; 1975) by looking at the expression of mouse mitochondrial DNA in minicells. This genome has a size of only  $10 \times 10^6$  and can be cloned in its totality, thus providing an opportunity for studying transcription and translation of a complete eukaryotic genome in a prokaryotic system, in contrast to previous studies which only involved genome fragments.

Covalently closed, circular mtDNA was partially digested with *EcoRI* restriction endonuclease, (in order to obtain linear molecules broken at only one of the two *EcoRI* sites on mouse mtDNA), and annealed with *EcoRI*-digested pSC101 plasmid DNA; after ligation, the DNA mixture was used to transform *E. coli* cells which were grown in the presence of tetracycline, to allow selection of cells transformed by the antibiotic resistance-carrying plasmid. The total closed, circular DNA population was then isolated from transformed cells and fractionated by sucrose gradient centrifugation. The 44S DNA molecules, corresponding to a molecular weight of  $16 \times 10^6$ , and therefore to the sum of mtDNA plus plasmid DNA, were amplified by a second cycle of transformation. Four different chimaeric molecules were recognised by heteroduplex analysis and by degradation with *HindIII*, a restriction enzyme having three sites on mtDNA and one (practically coinci-

dent with the *EcoRI* site) on pSC101. These molecules differed in the mtDNA *EcoRI* site used for plasmid insertion and in the relative orientation of plasmid and mtDNA.

MtDNA was then isolated from *EcoRI* digests of chimaeric plasmids by centrifugation in CsCl-netropsin density gradients, and separated into its heavy and light strand by centrifugation in alkaline CsCl. RNA labelled for 30 min with <sup>3</sup>H-uridine, obtained from minicells carrying chimaeric plasmids showed a continuous size distribution, as if unstable messenger-like RNA was being isolated in different stages of synthesis and degradation. In addition, most of the 30 min- and 3 min-labelled RNA hybridised to the L-strand, whereas little, if any, hybridised to the H-strand. Since the preferential transcription of the L-strand is not influenced by the relative orientation of pSC101 and mtDNA in the chimaeric molecules, it is improbable that transcription of the L-strand depended on a pSC101 promoter and read through at the mtDNA-pSC101 junction. Thus, one or more signals located within the L-strand are, indeed, recognised by the *E. coli* RNA polymerase. This does not, however, establish any structural identity between these sites and the promoter regions used inside functional mitochondria. In fact, as stressed by the authors, the opposite view is favoured by two lines of evidence. *In vivo*, mitochondrial rRNAs and most tRNAs and mRNAs are transcribed from the H-strand, only three tRNAs and one mRNA originating from the L-strand (Attardi *et al.*, in *The Biogenesis of Mitochondria*, 9, edit. by Kroon and Saccone, Academic Press, 1974). *In vitro*, mitochondrial RNA polymerase preferentially transcribes the H-strand (Wu and Dawid, *J. biol. Chem.*, **249**, 4412; 1974), whereas *E. coli* RNA polymerase preferentially transcribes the L-strand (Tabak and Borst, *Biochim. biophys. Acta* **217**, 356; 1970; Dawid, *Devl Biol.*, **29**, 139; 1972).

The total incorporation of <sup>3</sup>H-leucine into protein in minicells carrying the four types of chimaeric plasmids was found to be 2-4 times higher than that obtained from minicells containing pSC101 alone. The label was present in four polypeptides ranging in molecular weight from 2,500 to 5,000; four of the five pSC101-determined proteins were also seen. The protein synthetic pattern observed suggests that translation occurred from RNA transcripts of mtDNA; it differed, however, from that observed in mouse mitochondria, in which case seven proteins ranging in molecular weight from >40,000 to <10,000 were detected.

Among the additional findings of Chang *et al.*, one is of particular

interest, namely that the mtDNA origin of replication is not used at a significant frequency in chimaeric plasmids. In fact, all the *EcoRI* fragments originating from them and having the electron microscopic appearance of replicative intermediates had the length of pSC101; furthermore, their replication origins mapped in positions known to be the pSC101 replication origin.

To sum up this remarkable work, it has been demonstrated for the first time that transcription of eukaryotic DNA in prokaryotes can actually start at signals located on the eukaryotic DNA itself instead of being the result of read through from the plasmid; such transcripts, which appear to be translated in minicells, do not seem, however, to start at natural promoter sites on mtDNA and, therefore, do not correspond to any of the natural transcripts of the mitochondrial genome. This conclusion, which may, perhaps, disappoint the proponents of the prokaryotic origin of mitochondria, suggests that only a mitochondrial RNA polymerase is able to recognise mitochondrial promoters. In this case, the successful expression of eukaryotic genes in prokaryotes may need selective resection of their control elements, which should be replaced by those of plasmid or phage vectors (Murray *et al.*, *Xth FEBS Meeting*, **38**, 193; 1975). It should be recalled, however, that most of the mitochondrial transcripts are, in fact, derived from the processing of the single RNA copy of the H-strand (Aloni and Attardi, *J. molec. Biol.*, **70**, 363, 1972). Such a situation, implying the presence of a single promoter on the H-strand, makes animal mtDNA an unfavourable model to test its faithful transcription in minicells and leaves the door open, for the time being, to some optimism over the possibility of correct expression of eukaryotic genes in prokaryotes. An encouraging result along this line is the very recent finding by K. Struhl, J. Cameron and R. Davis of Stanford University of complementation of a *E. coli* *hisB* mutation by a yeast DNA fragment inserted into  $\lambda$  DNA. □

## B chromosomes in grasshoppers

from John Bishop

B CHROMOSOMES are chromosomes which appear in some but not in other members of a species, and as such are clearly not necessary for the survival of

individuals. The designation B indicates a supernumerary chromosome, in contrast to the A chromosomes, the representatives of the normal complement.

B chromosomes occur in many species of animals and plants. They are heterochromatic, do not pair with A chromosomes, and have no simple Mendelian effects upon the phenotype. They have been observed, however, to have very sharp clinal distributions which suggest a greater tolerance of B chromosomes under more favourable environmental conditions. The evolutionary significance of B chromosomes is elegantly explored in a paper by Hewitt in *Chromosomes Today* (4, edit. by Wahrman and Lewis, Wiley, New York, 1972).

Several studies have pointed to homologies between different B chromosomes and autosomes, centric fragments and X chromosomes. This suggests the exciting possibility that B chromosomes might represent one of the routes by which the genomes of eukaryotes change radically in informational content to take advantage of changing environmental opportunities. The B chromosome, being heterochromatic and therefore at least largely inactive, would be freed from selective constraint; but could be called on again (perhaps randomly) when and if it fulfilled a newly required function.

Because of this, it was both surprising and disquieting when Gibson and Hewitt (*Nature*, **225**, 67; 1970; *Chromosoma*, **38**, 121; 1972) suggested that the incidence of B chromosomes in the grasshopper *Myrmeleotettix maculatus* was correlated with the occurrence of a DNA satellite which accounted for as much DNA as the B chromosome(s) could be expected to comprise, if not more.

A satellite DNA is one with a different base composition and therefore a distinct buoyant density from the bulk of the DNA (the main-band DNA). Characteristically, a satellite DNA consists of very many similar short sequences (less than 20 nucleotide pairs) arranged tandemly in long continuous stretches (greater than 30,000 nucleotide pairs). On the other hand main-band DNA is largely composed of non-repetitive sequences intimately interspersed with repetitive sequences that have a much lower repetition frequency than satellite DNA.

If B chromosomes contained DNA that had such a degree of disparity with the A chromosome DNA then it would seem unlikely that the two were in any way related. This would very much weigh against the idea that B chromosomes are in the mainstream of the generation of new genetic function. If this were general, it would close, in eukaryotes, an enticing door which has only more recently

In liquid crystals long molecules are aligned parallel to one another but are disordered and free to move in the plane perpendicular to their long axis. The ends of the molecules may all be in register (smectic) or not (nematic), with all the chains parallel, or in register with successive layers of molecules tilted in different directions (cholesteric). It has always seemed likely that some synthetic polymers might form liquid crystals but until recently there has been no example of this in a pure polymer. Liquid crystal formers have a characteristic melting behaviour. On heating there is a change from crystal to liquid crystal with a large heat of transition followed a few degrees later by a transition with a small heat to the liquid. One should also be able to observe the partial ordering by X-ray diffraction and the molecular motion in the liquid crystalline phase by NMR.

Beatty and co-workers at Xerox (*Macromolecules*, **8**, 547; 1975) have now described an almost perfect example of such melting behaviour in polydiethylsiloxane. Calorimetry and X-ray diffraction provide supporting evidence and restricted molecular motion was observed by NMR and dielectric measurements. In other respects polydiethylsiloxane is a normally behaved semi-crystalline polymer and the amorphous regions of the structure seem unaffected by

the transition in the crystalline parts. Polysiloxane chains are quite flexible although it would seem more reasonable to expect liquid crystalline phases in stiff chains, where the inter-chain bonding could loosen at lower temperatures than those at which the chains could become free to rotate internally.

The morphology of the liquid crystalline phase and the mechanism of the transition from liquid to liquid

## Polymeric liquid crystals

from Paul Calvert

crystal and from liquid crystal to crystal in terms of chain folding and spherulite formation have yet to be elucidated. The work of Price and Stein (*J. Phys. Chem.*, **77**, 396, 399, 409; 1973) on the crystallisation kinetics of cholesteric liquid crystals provides a starting point for considering what is expected in the polymeric system.

It is possible that other polymer liquid crystalline phases have been overlooked because of their narrow temperature range. Beatty mentions a series of atactic stiff chain acrylates which have similar X-ray diffraction and melting behaviour to liquid crystals. These materials, however,

do not form true crystals and probably fit better into the grey area between ordered glasses and disordered crystals. A better example is the phase observed to form at high pressures in polyethylene by Bassett (*J. appl. Phys.*, **45**, 4146; 1974). This shows the same behaviour as polydiethylsiloxane though Bassett favours a disordered hexagonal crystal phase at present. He suggests that this phase is the cause of extended chain (large) crystal formation in polyethylene at high pressures. If this is a liquid crystal phase one could expect that extended chain crystals will also grow from polydiethylsiloxane.

There are some examples of solutions of polymers which form liquid crystals. Stiff chain aromatic polyamides in solution in acids form liquid crystals at about 10% polymer. Poly- $\gamma$ -benzyl-L-glutamate in dichloromethane is similar (Duke and DuPré, *Macromolecules*, **7**, 374; 1974; Hines and Samutski, *Macromolecules*, **6**, 793; 1973), on the face of it, however, these solution systems are a different case from pure polymer.

This would be a good time for polymer physicists to predict the arrangement of chains in the liquid crystalline phase and to predict which other polymers should form liquid crystals. Current theories of polymer crystallisation seem rather *ad hoc* and a successful prediction could add respectability.

been opened towards the prokaryotes (Wallace and Morowitz, *Chromosoma*, **40**, 121; 1973).

The situation reported for *M. maculatus* was not, however, observed in other organisms (see for example Chilton and McCarthy, *Genetics*, **74**, 605; 1973). Furthermore, the *M. maculatus* satellite had most unusual properties: not only did it contain highly-repetitive DNA, as do all DNA satellites, but most of it consisted of DNA sequences which, as far as the satellite was concerned, were unique. This was, so to speak, a unique observation.

From the same group in Norwich, but with different authorship, there came recently a confirmation of the correlation between B chromosome content and the occurrence of satellite in *M. maculatus* (Wilmore and Brown, *Chromosoma*, **51**, 337; 1975). This study pointed up a most interesting coincidence: the buoyant density of the *M. maculatus* satellite is identical to that of *Paramecium aurelia* DNA, to the third decimal place. It also has much the same analytical complexity (compare Gibson and Hewitt, *Chromosoma*, **38**, 121; 1972; and Cummings, *Chromosoma*, **53**, 192; 1975).

Yet more recently, Dover and Henderson (*Nature*, **259**, 57; 1975) have reopened the issue by examining some of the same populations of *M. maculatus* previously examined by Gibson and Hewitt. They did indeed find B chromosomes, but found no satellite, even in the presence of actinomycin D. In fact, grasshoppers as a group seem to be unusually free of satellites (Wilmore and Brown, *op. cit.*, 1975).

The unusual properties of the satellite observed by Gibson and Hewitt offer a conceivable solution to the problem. It shows the characteristics of an entire genome, rather than those common to other eukaryotic satellites, and furthermore, it is uncannily like a ciliate genome. It seems possible, then, that the DNA of some grasshoppers is contaminated by that of a ciliate, perhaps a ciliate parasite. It is not by any means inconceivable that the distribution of such a parasite would correlate, perhaps loosely, with that of B chromosomes.

Whatever the eventual explanation it would seem that B chromosomes may soon return securely to their former place as potential progenitors of genetic diversity in eukaryotes. Perhaps this

is one instance in which a negative finding could ultimately outweigh a positive one. □

## The early Earth

from Lynn Margulis

The second College Park Symposium—Chemical Evolution in the Precambrian—was held at the University of Maryland on October 29–November 1. It was sponsored by NASA and the NSF and organised and chaired by Professor Cyril Ponnamperna, Laboratory of Chemical Evolution, Department of Chemistry, University of Maryland. The proceedings will be published by Academic Press, New York, jointly with last year's College Park Symposium on the giant planets.

WHAT were conditions like 4 thousand million years ago? Despite enormous diversity in training, formal back-



ground, methods and scientific approaches amongst the participants agreement on certain basic concepts did emerge at the meeting. V. Rama-Murthy's (University of Minnesota) description of the role of the iron-nickel-lighter substances in the formation of Earth's core and G. Wetherill's (Carnegie Institution of Washington) time scale and planetological-geological framework for the early Precambrian seemed generally accepted. The early planetary period began about 4.43 billion ( $10^9$ ) years ago (bya) when heavy bombardment of the Earth by Solar System debris occurred and is thought to have gone on until about 3.88 bya. From this time until the present we have at least an intermittent but continuous rock record. A middle period of meteoritic bombardment is recognised between 2.85 and 2.6 bya which corresponds to the principal period of igneous activity on the Moon and is thought to have also been marked by volcanism on the Earth. These events "reset" most of the older geochronological clocks. Challenging previous work by Goldich claiming an age of 3.8 byr, Wetherill and his colleagues claim that the Earth's most ancient rocks reliably aged to date have been found on the coast of Labrador and in Greenland. These old gneisses were probably contiguous some 3.62 bya. Only slightly younger than the oldest rocks themselves is the oldest evidence for life in sediments. J. Henderson (Geological Survey of Canada) described sialic crustal material from the Archaean above a granitic basement about 3.0 byr old (Slave Structural Province, Canada). The oldest presumptive evidence for life here is based on black carbonaceous shales and those laminated heros of the Precambrian stage—stromatolites. Thus, Canada is beginning to compete for attention with the remarkable ancient sediments of the lower Precambrian in southern Africa's Swaziland System.

Whereas the Isua Series (the ancient rocks of southwestern Greenland) of moderately metamorphosed sediments yield a rather dull carbon profile with none of the finely dispersed kerogen-like materials typical of younger rocks, only traces of methane and so forth and rather lunar-like with respect to paucity of carbon, the South African (Swaziland Supergroup) Onverwacht and Fig Tree sediments are much richer. B. Nagy (University of Arizona) and his colleagues found aromatic hydrocarbons (by ozonolysis) and, in the Fig Tree, long chain normal alkanes as well. In the organo-geochemical analyses of the Transvaal stromatolite kerogen considerably more hydrocarbon, oxygen, nitrogen and sulphur compounds are found in the

sediments (2.3 byr). Of course interpretations are risky but it is highly possible that life arose between 3.6 and 3.0 bya and that for a while both chemical and biological evolution continued together. (The possibility that the Fig Tree microspheres are not biological but rather prebiotic chemical fossils was well explored by J. Wm. Schopf (University of California, Los Angeles).)

Although alternative interpretations are possible it seemed that the participants at the meeting would tend to endorse the following scheme. Shortly after the Earth stabilised life evolved and went into Darwinian motion and by 2.7 bya (in Canada and South Africa) bacteria and blue green alga had evolved, diversified, and were flourishing as demonstrated by L. A. Nagy's (University of Arizona) Transvaal microfossils (2.3 byr old) of both coccoid and filamentous forms.

For the history of the atmosphere both J. C. G. Walker (National Radio Observatory, Arecibo, Puerto Rico) and R. Siever (Harvard University) presented arguments that degassing from the interior of the Earth probably occurred during the earliest accretion phase (4.55–4.33 bya) and that most other gaseous emission since then has been recycling rather than accumulating. Everyone seemed to agree with Siever that the earliest Archaean atmosphere was anoxygenic but that in other ways (with respect to weathering, composition and total pressures) the atmospheric-surface processes were not very different from those today. Neither Siever nor Walker (nor anyone else) argued that the Archaean atmosphere containing hydrogen, methane and ammonia was highly reducing. Nor did that seem to bother anyone. For example, C. Folsome (University of Hawaii) demonstrated that he could make perfectly good organic microspheres in Urey-Miller type electrical discharges with  $\text{CO}$ ,  $\text{CO}_2$  and  $\text{N}_2$ . In this system, although methane could replace  $\text{CO}_2$  as a carbon source, it was not required for the abiotic generation of simple organic "formed bodies". There was agreement with P. E. Cloud Jr's concept that the atmospheric transition from anoxygenic to oxygenic occurred some 2 bya. A common surface mat blue green alga (for example *Lyngbya* sp.) survives potentially lethal quantities of ultraviolet for days when afforded protection by "matting" (inoculum size) and/or sodium nitrate or nitrite in the medium (Rambler, Boston University) so it is likely that even before the appearance of stratospheric ozone, algal mat communities could cope with ultraviolet light.

T. Hoering (Carnegie Institution of Washington) inspired a good deal of

enthusiasm with his preliminary data on hydrogen isotope fractionations found in fossils and presumably generated biologically. Whatever the real cause of these fractionations, deuterium/hydrogen ratios promise to serve us well as a complement to carbon isotope work.

Although everyone agreed that the Precambrian was the "age of the prokaryotes" there was no consensus concerning the time of first appearance of eukaryotic cells. Primary sequence data on primitive proteins such as ferredoxin (D. O. Hall, Kings College, London) and cytochromes (Cohen and M. Dayhoff, Georgetown University) are proving helpful in reconstructing the evolutionary past. Extrapolations from accepted point mutation rates in proteins and RNAs do suggest that Schopf's evidence for the appearance of nucleated algae by the time Bitter Springs deposits were made ( $850 \pm 100$  Myr) is very reasonable.

But that rich fossil assemblage may indeed be evidence not for eukaryotes but for the acme of prokaryotic development. Schopf led a lively discussion in which he maintained that "nucleated organisms were extant prior to  $850 \pm 100$  Myr and that the lineage may well have been established as early as  $1,400 \pm 100$  Myr ago." Some of our results with artificial "fossils" (using ethyl silicate) and Knoll and Barghoorn's observations on partially degraded blue green algae counsel caution in interpreting the Bitter Springs fossil biota as containing *bona fide* remains of chlorophyte or rhodophyte algae.

The Proterozoic (2 bya) Gunflint stromatolites and microfossils were discussed excitedly in terms of their modern counterparts. *Metallogenium* (a manganese and iron-oxidising microbe discovered by the Russians) bears a striking resemblance to the Gunflint genus *Eoastirion* (E. S. Barghoorn, Harvard University). Everyone welcomed B. Siegel's (University of Hawaii) presentation on newly collected cultures of the enigmatic *Kakabekia barghoorniana*, the microbe that so resembles the Gunflint fossil *Kakabekia umbellata*. Although new data is available on this organism, which has now been collected from diatom-rich cold alkaline places all over the world, the details of its life cycle and culture requirements remain unknown.

Attacking the site of the origin of life itself straight on L. Onsager (University of Florida, Miami) discussed interfacial polymerisation processes that might have occurred at the geochemically derived "primordial oil slick", which, theoretically at least, was conveniently supplied by M. J. Dwyer (University of Philadelphia).

# articles

## Influence of ancient solar-proton events on the evolution of life

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*There is mounting evidence that past extinctions of faunal species have occurred in near coincidence with reversals in polarity of the geomagnetic field. Could the link lie in catastrophic depletions of stratospheric ozone caused by solar-proton irradiation over a reduced geomagnetic field?*

We discuss here a new mechanism that could explain the apparent correlation between faunal extinctions and reversals of the geomagnetic field more satisfactorily than other mechanisms proposed so far.

Uffen has suggested<sup>1</sup> that the surface of the Earth could be exposed to abnormally high fluxes of radiation during the periods of reversal of the polarity of the geomagnetic field (see ref. 2). During these geologically brief periods, the shielding effects of the magnetic field would be removed, or at least severely weakened, allowing galactic cosmic rays to have access to the entire Earth, instead of merely to high-latitude regions, and also causing the radiation belts to precipitate. Uffen proposed<sup>1</sup> that this enhanced level of radiation could lead to increased mutation rates among living species, thereby explaining sudden appearances and disappearances of species from the palaeontological record.

His suggestion has since been examined<sup>3,4</sup> and it is now generally felt that the effect on living species would probably be insignificant. Nonetheless, there is mounting evidence that a correlation does exist between major faunal extinction and geomagnetic polarity reversals. The validity of this correlation in fairly recent geological time seems to have been well established by studies of fossil species of radiolaria (single-celled, marine microorganisms). Using deep-sea cores obtained from a variety of locations, Hays<sup>5</sup> has determined that during the past 2.5 Myr eight species became extinct, and that six of those disappeared in near coincidence with polarity reversals.

Hays has estimated that the probability of a chance coincidence between faunal extinctions and polarity reversals is less than 0.002 (see also refs 6 and 7). Going back still further in time, there is more tenuous evidence of a general correlation between polarity reversals and extinctions of both marine and terrestrial fauna<sup>8,9</sup>. The establishment of a clear case for the radiolarian extinctions has only been possible through a fortunate combination of circumstances that allows the distinctive fossilised siliceous skeletons of the radiolaria to be studied in direct relationship to the geomagnetic record in the same deep-sea cores.

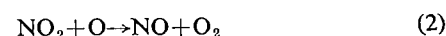
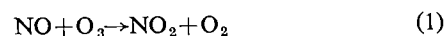
Hays' work<sup>5,7</sup> has shown that individual species can survive through several polarity reversals, though they may often

decrease in numbers at the time of a reversal, before finally disappearing. This suggests that the harmful effects accompanying a polarity reversal, whatever they may be, form only one component of the total environmental stress on a given species. Extinction may result from the addition of this factor to others that are already subjecting the species to severe stress at the time of the reversal.

Other mechanisms linking changes in the geomagnetic field with effects on living organisms have been proposed, include climate changes<sup>10</sup> and direct magnetic effects on growth<sup>5,9</sup>, among others. None has yet received acceptance, however.

Many solar flares generate fluxes of energetic particles, mostly protons, at the Earth. These events occur sporadically, though their frequency of occurrence is strongly dependent on the general level of solar activity manifested in the 11-yr solar cycle. The energies of the individual particles cover a very wide range, from roughly 10<sup>4</sup> eV to over 10<sup>9</sup> eV, and the intensities and spectra are extremely variable from event to event. Though the fluxes of particles capable of reaching the lower atmosphere or ground level (that is, those with energies in excess of 10<sup>9</sup> eV) are generally small and of short duration, however, very substantial fluxes, often persisting for many days, reach the stratosphere during many events.

Crutzen *et al.*<sup>11</sup> have shown that the ionisation produced in the stratosphere by these particles leads to the formation of nitric oxide in large quantities through the dissociation and the dissociative ionisation of N<sub>2</sub>, followed by the reaction of N atoms with oxygen. A few intense events a year can produce NO in amounts comparable with those produced by the main known source (the oxidation of N<sub>2</sub>O), and considerably larger than those produced by galactic cosmic rays. Ozone is catalytically destroyed by NO (ref. 12) chiefly through the pair of reactions



thus directly affecting the efficiency of the ozone shield that protects the surface of the Earth from potentially harmful solar ultraviolet radiation. Although each solar-proton event lasts only a few days, the lifetime of NO in the stratosphere is long, and its effect on the ozone layer is likely to persist for several years.

At present, the geomagnetic field effectively guides the solar protons towards high geomagnetic latitudes, and for most practical purposes the direct effects on the atmosphere are confined to geomagnetic latitudes higher than about 60°. The long lifetime of NO ensures that it will be spread over the Earth by horizontal transport, but it will be diluted by a geometrical

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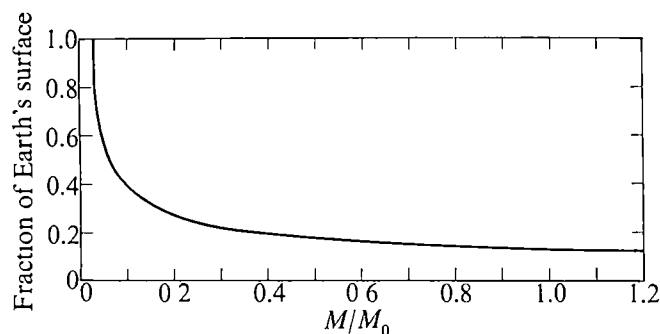
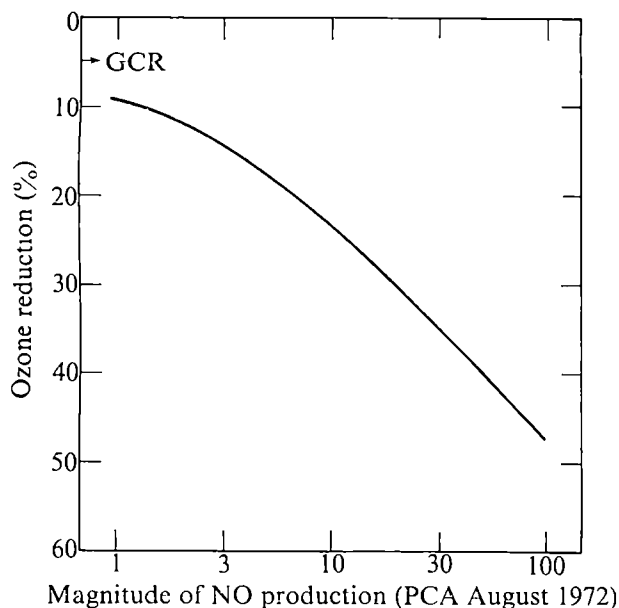


Fig. 1 An approximate estimate of the fraction of the global stratospheric surface exposed to solar protons of 30 MeV energy as the Earth's dipole moment,  $M$ , is changed from its present value  $M_0$ .

factor of about 7, corresponding to the fraction of the Earth lying at latitudes higher than  $60^\circ$ . During polarity reversals, however, the dipole component of the geomagnetic field probably weakens or disappears for periods of a few thousand years<sup>13</sup>, allowing both solar protons and galactic cosmic rays access to much larger areas of the Earth, and leading to much more severe effects on the ozone shield than are possible at present. Biological species that had evolved over the preceding several million years of geomagnetic stability may be unable to survive the harsher ultraviolet environment, and would presumably be replaced by other species with more adaptability. It is worth noting that many contemporary simple forms of aquatic life seem to be intolerant of enhanced ultraviolet radiation in the 300-nm wavelength range<sup>14</sup>, which can penetrate water to depths of several metres<sup>15</sup>.

This mechanism is related to one proposed by Ruderman<sup>16</sup>, who suggested that the sporadic occurrence of supernovae in the past might have subjected the Earth to intense fluxes of radiation, resulting in a removal of the ozone shield through the catalytic action of NO. This mechanism should certainly be considered, but it cannot explain the observed correlation between major faunal extinctions and polarity reversals. The solar-proton hypothesis, on the other hand, provides at least a tentative explanation of this mysterious relationship.

Fig. 2 Fractional depletion of the total content of the ozone column of the stratosphere produced by intense solar-proton events in the absence of a geomagnetic field. The events are assumed to have the same spectrum as that of the August 1972 event, and the abscissa indicates the particle flux in units of the August 1972 flux. GCR, the calculated depletion produced by the continuous action of galactic cosmic rays.



Besides undergoing polarity reversals, the intensity of the geomagnetic field varies on a time scale of thousands of years. There is no evidence for any biological effects related to this variation, and a possible explanation is illustrated in Fig. 1. This shows the fraction of the global atmospheric surface accessible to protons of 30 MeV energy as a function of the geomagnetic dipole moment, normalised to unity at its present value. Since 30 MeV is roughly the minimum energy needed to reach the stratosphere, this quantity represents the fraction of the stratosphere that is exposed to the full available solar-proton flux. The calculation is approximate, based on a simple dipole field, in which the Störmer theory of geomagnetic cutoffs is applicable, with a  $5^\circ$  correction applied to take account of the distortion of the outer regions of the field by the solar wind<sup>17</sup>. Figure 1 shows that the dipole moment must be reduced by a factor of 10 or more before the area directly affected by solar protons is greatly increased. Evidence from rock magnetisation studies indicate that the field intensity does decrease by an order of magnitude during polarity reversals, but that it does not completely disappear. The remaining field is likely to comprise

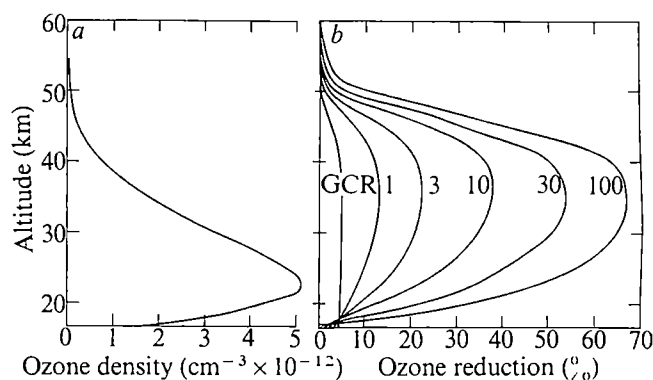


Fig. 3 *a*, Calculated ozone height profile for normal conditions, *b*, fractional depletion produced by solar-proton events of the indicated magnitudes (relative to the event of August 1972), and by galactic cosmic rays (GCR).

mainly non-dipole components whose effect on energetic particles is impossible to estimate. It is likely, however, that non-dipole fields will allow freer access than would dipole fields of similar strength. We assume here that the field actually disappears during polarity reversals.

Solar-proton events have been studied only during the past 15–20 yr. Based on the accumulated evidence, it is difficult to estimate the maximum intensity that an event may reach during an episode of polarity reversal lasting perhaps a thousand years or more. The most intense event known was that of August 1972, during which about  $2.5 \times 10^6$  erg  $\text{cm}^{-2}$  entered the high-latitude atmosphere in the form of protons with energies greater than 30 MeV, which penetrate into the stratosphere. It does not seem that events one or two orders of magnitude more intense than that of August 1972 can be ruled out when the observation period is lengthened to more than 1,000 yr. The quantity of NO that can be produced during extremely intense events is not unlimited, however, since the reaction



which re-creates  $\text{N}_2$  from dissociated N atoms, assumes major importance when the NO concentration is high.

Figure 2 shows our estimate of ozone reductions resulting from the increased stratospheric production of NO by the continuous action of galactic cosmic rays and by sporadic solar-proton events occurring during polarity reversals. It is assumed that during these periods the atmosphere is otherwise unchanged from its present state. The calculations were carried out using a one-dimensional, time-dependent, photochemical model, which is an extension of a model developed by one of us (P.J.C.), and which will be described elsewhere. The production rates

of NO from galactic cosmic rays were obtained from a study by Brasseur and Nicolet<sup>18</sup>. The magnitudes of the solar-proton events are here measured in terms of the August 1972 event, but it must be remembered that in reality different events differ greatly in their particle energy spectra as well as their fluxes. The numbers probably represent underestimates of the effects, since the conditions of the model calculations were chosen to simulate the effects of the solar protons at low latitudes. As NO production from the oxidation of N<sub>2</sub>O is a maximum at low latitudes, the contribution of NO from solar-proton events relative to that from N<sub>2</sub>O oxidation is larger on a global scale than is indicated by the present model.

The calculations show that very substantial ozone reductions must accompany intense solar-proton events during polarity reversals, and also that the maximum effect is reached 1–2 yr after the event, and the time required for the atmosphere to recover to its initial condition is almost 10 yr. Even in the absence of intense solar-proton events, the ozone content will be reduced by 4.3% by the continuous action of galactic cosmic rays.

The altitude dependence of the ozone reduction is shown in Fig. 3, which indicates that the main effect occurs well above the maximum of the ozone layer, at least near the time of peak reduction. Figure 4 shows the percentage change in the solar heating rate attributable to the absorption of ultraviolet and visible radiation by O<sub>3</sub> and NO<sub>2</sub>. It may, incidentally, be concluded that changes in the Earth's climate may result from intense solar-proton events occurring during polarity reversals. In fact, during very intense events enough NO<sub>2</sub> could be formed in the stratosphere to affect significantly the penetration to ground level of visible radiation in the 400–500-nm range, where NO<sub>2</sub> absorbs radiation rather strongly.

We have shown that intense solar-proton events occurring during polarity reversals may cause large reductions in the atmospheric column content of ozone. The effect of such depletions on living organisms is difficult to estimate, but Fig. 5 gives a rough indication of the magnitude of the effects. Assuming a thickness of the ozone layer of 0.3 cm, and adopting the solar fluxes of Ackerman<sup>19</sup>, the total number of photons incident per cm<sup>2</sup> during an equinoctial day at the Equator has been calculated as a function of wavelength in the 270–320-nm range. Fluxes at each wavelength have been multiplied by a 'relative biological effectiveness' factor<sup>20</sup>, based on the response of protein and nucleic acids to ultraviolet radiation. The calculations show that during a polarity reversal a solar-proton event of the same magnitude as the August 1972 event would increase the effective dose by 15%, and that events 10 and 100 times more intense would increase the dose by 55% and 160%, respectively. Such increases would inevitably have some effect on simple freshwater

Fig. 4 A, Calculated heating rate under normal conditions attributable to the absorption of solar radiation by: a, O<sub>3</sub>; and b, NO<sub>2</sub> ( $\times 10^3$ ). B, Fractional changes attributable to intense solar-proton events and galactic cosmic rays during polarity reversals. The increased heating rates at the lower levels are attributable to the increased penetration of solar ultraviolet radiation when the overlying ozone is depleted.

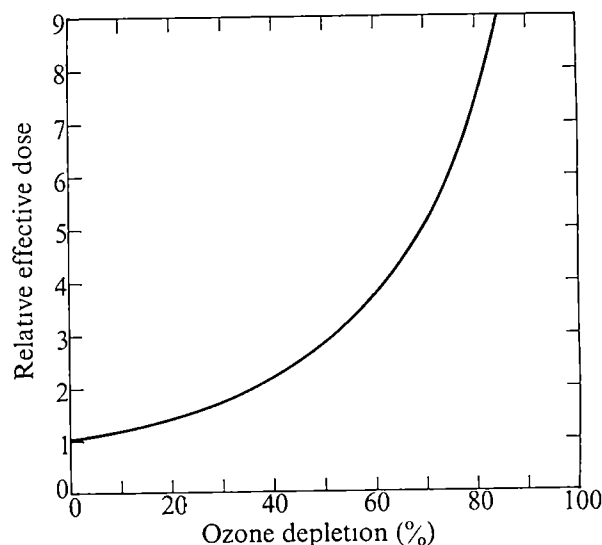
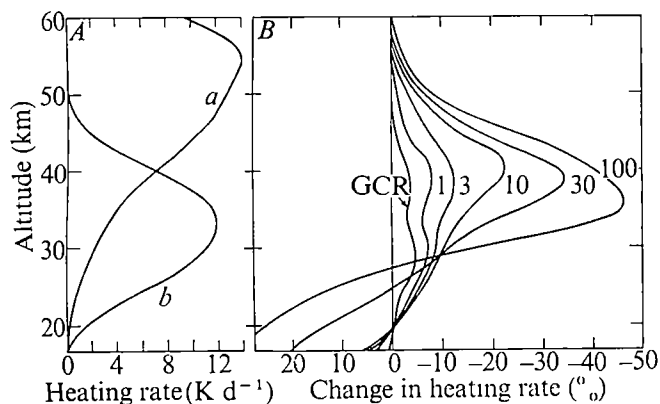


Fig. 5 The relative effective dose of ultraviolet radiation received on an equinoctial day at the Equator as a function of the depletion in stratospheric ozone.

microorganisms, many of which at present seem to be living close to their maximum tolerance of ultraviolet radiation<sup>14</sup>. The effect on marine organisms is unknown, and will depend on such factors as the length of time spent in the upper part of the euphotic zone, where the ultraviolet dosage will be highest, and on the effect of the increased ultraviolet light on phytoplankton. The total effect on the organisms, however, is certainly an increase in environmental stress, which may, as already mentioned, be sufficient to lead to extinction.

Though the radiolarian extinctions discussed here may have resulted from the addition of one new stress factor to others that existed already, it is perhaps worth speculating that the mechanism we have described may have had the dominant role in some of the massive faunal extinctions that have occurred in the distant past. For example, roughly one-third of all living species became extinct at the close of the Cretaceous, which was a period marked by a resumption of polarity reversals, following a very lengthy period of normal polarity<sup>21</sup>.

In conclusion, it seems that current concern about possible anthropogenic destruction of stratospheric ozone may be well founded, since it is possible that major ozone depletions occurring in the distant past have had a profound effect on the development of life as we know it.

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# Thickening of the Ross Ice Shelf and equilibrium state of the West Antarctic ice sheet

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*Data from the south-east quadrant of the Ross Ice Shelf, Antarctica indicate that, near the grounding line, the ice shelf is growing thicker by almost  $1 \text{ m yr}^{-1}$ . This thickening rate implies an advance of  $1 \text{ km yr}^{-1}$  of the grounding line between the West Antarctic ice sheet and the Ross Ice Shelf. It can be reconciled with independent evidence for current thinning of the West Antarctic ice sheet if the latter is a delayed response to an earlier retreat of the ice shelf grounding line. The high rates of ice shelf thickening may be due to an increase in the resistance to ice shelf movement caused by localised ice shelf grounding following isostatic uplift of the sea bed.*

Most of the West Antarctic ice sheet (Fig. 1) rests on rock that is below sea level now and would still be below sea level if the ice sheet were removed and complete isostatic rebound occurred. Mercer<sup>1</sup> has suggested that such an ice sheet could disintegrate rather rapidly if the surrounding ice shelves were removed, and Hughes<sup>2</sup> noted that the surface profile of the West Antarctic ice sheet (WIS) was not an equilibrium profile and suggested that the ice sheet may currently be disintegrating. Here I shall use measurements made by the Ross Ice Shelf Project (RISP) (refs 3, 4 and H. B. Clausen and W. Dansgaard, personal communication) to examine the state of equilibrium of parts of the Ross Ice Shelf (RIS). I shall correlate this information with other available data to evaluate current behaviour of the WIS.

## Data

The results from RISP are summarised in Fig. 2 where strain rates, accumulation rates, and ice velocities are plotted against distance along the flow line that passes through the site of a proposed bore hole through the ice shelf and into the sea bed. The path of the flow line across the ice shelf was deduced from measurements of ice velocity and surface strain rate<sup>3</sup>. It enters the ice shelf from ice stream 'B' (Fig. 1) and has been extrapolated on to the WIS assuming the ice to be moving perpendicular to the surface contours. The catchment area for ice stream 'B' is also shown in Fig. 1. Snow accumulation rates ( $A$ ) in the catchment area (Fig. 2) are from Bull<sup>5</sup>, and local ice velocities ( $V$ ) have been calculated assuming that ice movement exactly compensates upstream snow accumulation. Vertical strain rates ( $\dot{\epsilon}$ ) in this area were then calculated from the equation of continuity<sup>6</sup>

$$\partial H / \partial t + V \partial H / \partial x = A + B + H \dot{\epsilon} \quad (1)$$

where  $x$  is distance along the flow line,  $H$  is ice thickness,  $\partial H / \partial t$  is the rate of thickening of the ice sheet,  $\partial H / \partial x$  is the thickness gradient along the flow line,  $B$  is bottom freezing rate ( $A$  and  $B$  are expressed as depths of ice per unit time; on the WIS I have assumed that  $|B| \ll |A|$ ) and  $\dot{\epsilon}$  is negative for thinning. To calculate values of  $\dot{\epsilon}$  upstream of the grounding line, I have assumed that in this area  $\partial H / \partial t = 0$ . In the central part of the ice shelf  $A$  and  $V$  values have been interpolated

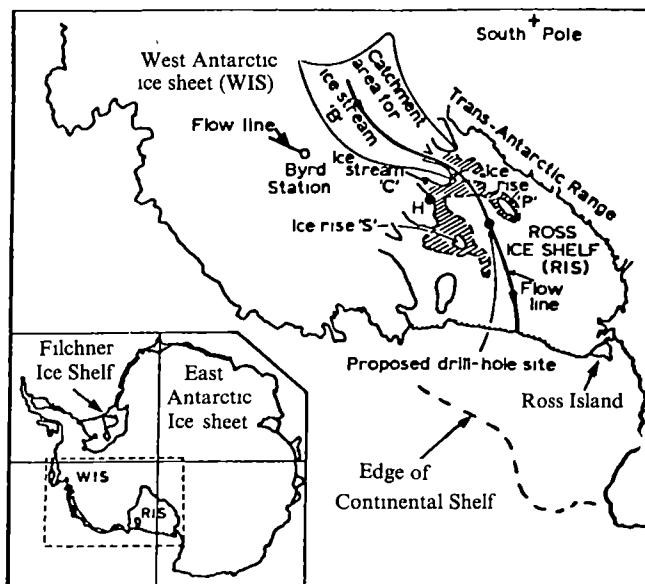
between the RIPS measurements and data from near the ice front<sup>7,8</sup>, and for this region I have assumed zero lateral strain, so that  $\dot{\epsilon} = -\partial V / \partial x$ . This assumption is probably valid near the ice front<sup>7</sup>, but further upstream there may be lateral compression because of converging ice streams. Ice thickness data<sup>4,8-10</sup> are most reliable for the ice shelf section of the flow line.

## Interpretation

The data shown in Fig. 2 has been used to calculate steady-state snow particle paths<sup>11</sup> from where they land on the surface to the point of exit from the ice shelf (Fig. 3). Ice velocity is assumed to be independent of depth, which is true for the ice shelf and is a good approximation for most of WIS, where ice movement is predominantly by bottom sliding<sup>12</sup>. The particle-path results give the relationship between age and depth at all points along the flow line, and Fig. 4 is a plot of age against depth at the site of the proposed drill hole, but inevitably, these ages can be regarded as only approximate. Furthermore, the steady-state assumption (that conditions along the flow line are invariant with time) is a rather drastic restriction. Indeed 10,000 yr ago the WIS probably covered the entire Ross Ice Shelf. Strain rates and ice velocities would then have been considerably smaller than those in Fig. 2. Lower strain rates imply a slower thinning of the annual layers and, even if snow accumulation was lower over a more extensive WIS, the deepest ice is probably younger than shown in Fig. 4.

Where the particle paths leave the base of the ice shelf, bottom melting must occur for the ice shelf profile to be time

**Fig. 1** Part of the West Antarctic ice sheet and the Ross Ice Shelf. In the shaded area, the sea bed is within 50 m of the ice shelf bottom; this entire region may be grounded within the next 200 yr.



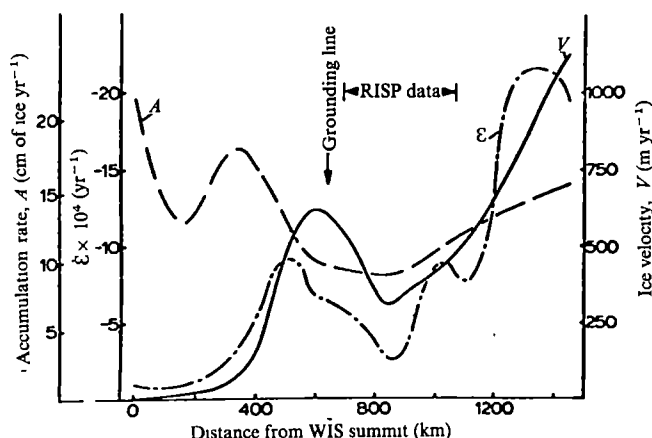


Fig. 2 Snow accumulation rate ( $\dot{A}$ ), ice velocity ( $V$ ) and vertical strain rates ( $\dot{\epsilon}$ ) plotted against distance along the flow line that passes through the proposed RISP drill-hole site.

invariant. Similarly, bottom freezing occurs where particles enter the base of the ice shelf. Figure 3 indicates that, for a steady state, appreciable bottom melting is taking place near the junction, or grounding line between the WIS and the RIS, with the bottom freezing nearer the ice front. We should, however, expect the opposite to be true with maximum bottom freezing near the grounding line. Indeed, observations of sediment laden ice near ice rise 'P' (ref. 13), where severe fracturing has probably overturned parts of the ice shelf, suggest that bottom freezing is taking place upstream of the ice rise. Consequently, we relax the steady-state assumption, and use equation (1) to study the non-steady-state behaviour of the ice shelf.

### Non-equilibrium behaviour

Equation (1) has been solved to give  $(\partial H/\partial t - \dot{B})$  for the ice shelf section of the flow line, and the results are given in Fig. 5. Upstream of the drill-hole site  $\dot{B}$  probably has a small positive value, but for simplicity we shall assume that  $\dot{B} = 0$ . Figure 5 then becomes a plot of the rate of ice shelf thickening  $(\partial H/\partial t)$  against distance from the grounding line and, within the limits of observation error,  $\partial H/\partial t$  decreases linearly from  $\sim +1 \text{ m yr}^{-1}$  at the grounding line to zero near the drill-hole site. An interesting effect of this linear decrease in  $\partial H/\partial t$  is that the magnitude of the thickness slope  $|\partial H/\partial x|$  (and thus, from equation (1), of  $\partial H/\partial t$ ) increases with time. Downstream from the drill-hole site the ice shelf appears to be in equilibrium with bottom melting within  $\sim 200 \text{ km}$  of the ice front, as is approximately depicted by the dashed curve. The large deviations from the dashed curve almost certainly arise from over-estimation of the relevant vertical strain rates, which were calculated assuming lateral strain to be zero. In reality there is probably lateral compression from the converging ice streams.

It is important to note that the high rates of ice shelf thickening shown in Fig. 5 near the grounding line were calculated solely from the RISP data and have an accuracy of  $\sim \pm 0.2 \text{ m yr}^{-1}$ . Although this is the only region where we have 'hard' data, the results shown in Fig. 5 are of particular interest since they imply an annual advance of the grounding line in this area of  $\sim 1 \text{ km}$ , and if the ice shelf continues to thicken at its present rate it will run aground on the bed rock, 100 km upstream of the drill-hole site (Fig. 3), in  $\sim 50 \text{ yr}$ . It should be stressed that such rapid thickening is not expected to be typical for the entire grounding line; indeed data from an area where the ice is moving by slow 'sheet flow' ( $H$  in Fig. 1) indicate that the local grounding line is almost stationary. Nevertheless, ice streams are responsible for most of the WIS drainage, and their present behaviour is a good indicator for the probable future behaviour of WIS.

### Interpretation

The high rates of ice shelf thickening near the grounding line are of considerable interest, particularly since other evidence<sup>14-17</sup> suggests that the WIS has thinned appreciably during the past 10,000 yr and may still be thinning. Equation (1) can be solved using measurements from Byrd Station (Fig. 1)<sup>2,12</sup> to give  $(\partial H/\partial t - \dot{B}) \approx -0.4 \text{ m yr}^{-1}$ .  $\dot{B}$  is probably  $< 10^{-2} \text{ m yr}^{-1}$  (ref. 12), so the ice at Byrd Station is currently thinning by  $\sim 0.4 \text{ m yr}^{-1}$ . Upstream from Byrd Station, the available data<sup>2,12</sup> indicate that the vertical strain rate drops, to give a thinning rate of  $\sim 0.1 \text{ m yr}^{-1}$  near the WIS summit.

Analysis of the concentration of microparticles in ice from a bore hole at Byrd Station gives estimates of age as a function of depth<sup>18</sup>. These can be used to derive historical vertical strain rates, and the record for the past 2,000 yr shows good agreement with the recently measured values. For  $\sim 25,000 \text{ yr}$  before 2,000 yr BP, however, vertical strain rates were between 5% and 10% of the present values upstream from Byrd Station. Probable temporal variations in snow accumulation cannot account for this large apparent reduction in strain rates before 2,000 yr BP. Instead, I suggest that a real increase in strain rates took place  $\sim 2,000 \text{ yr}$  ago. If we assume that present-day vertical strain rates ( $\dot{\epsilon} \approx -10^{-4} \text{ yr}^{-1}$ ) upstream of Byrd Station are typical for the past 2,000 yr we can estimate the 2,000 yr BP ice thickness as

$$H = H'/(1 + \dot{\epsilon})^{2,000} \quad (2)$$

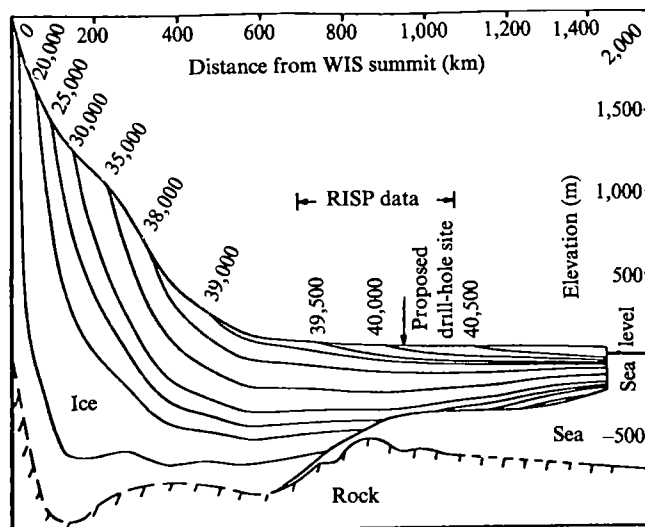
where  $H'$  ( $\sim 2,500 \text{ m}$ ) is the present average height above bed rock of the 2,000 yr BP ice surface, giving  $H \sim 3,050 \text{ m}$ , or  $\sim 350 \text{ m}$  thicker than the present ice sheet. This is in agreement with general conclusions from stable isotope analysis of ice from the Byrd Station bore hole<sup>14</sup> and from interpretation of temperatures measured in the bore hole<sup>15</sup>.

Near the summit of an ice sheet  $V\partial H/\partial x \rightarrow 0$  and probably  $\dot{B} \ll \dot{A}$  so equation (1) can be written:

$$\partial H/\partial t \sim \dot{A} + H\dot{\epsilon} \quad (3)$$

With the pre-2,000 yr BP vertical strain rate ( $\sim -10^{-5} \text{ yr}^{-1}$ ) equation (3) gives  $\partial H/\partial t \sim \dot{A} \rightarrow (\dot{A} - 0.03) \text{ m yr}^{-1}$ , for  $H = 0 \rightarrow 3,000 \text{ m}$ . The average value now of  $\dot{A}$  upstream of Byrd Station is  $0.17 \text{ m yr}^{-1}$  (ref. 19), and even assuming a significant

Fig. 3 Steady-state particle paths for the flow line that passes through the proposed RISP drill-hole site. The numbers at the point of origin of each flow line represent the time taken in years to pass each point for a snow particle deposited 20 km downstream from the WIS summit. Sea depth was measured in the RISP area<sup>4</sup> and near the ice front<sup>8</sup>.



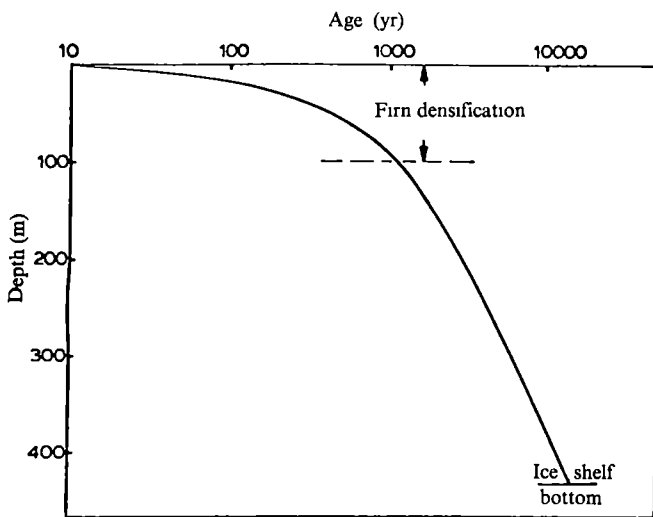


Fig. 4 A plot of age as a function of depth at the proposed RISP drill-hole site based on steady-state assumptions. Actual ice sheet behaviour implies that deeper ice is probably younger than shown

reduction in  $\dot{A}$  associated with more widespread glaciation we may conclude that the WIS summit was thickening continuously until  $\sim 2,000$  yr BP, when thinning commenced. This implies that the WIS failed to achieve an equilibrium state, and is compatible with the theory<sup>20</sup> that the WIS can exist only in a state of growth or shrinkage unless its outer margins reach to either a point where the sea has a critical depth or the edge of the continental shelf. It is important to note that the quantitative estimates of ice thickness and rates of thickening before 2,000 yr BP are very approximate, and improvement in their accuracy must await data from drill holes upstream from Byrd Station. It is, however, probably safe to conclude that the ice thickness at the WIS summit 2,000 yr BP was significantly greater than today, and that before 2,000 yr BP the ice sheet had been thickening for  $\sim 25,000$  yr. Moreover, analysis of microparticle concentrations in the Byrd Station bore hole indicates that the basal ice is  $\sim 27,000$  yr old, implying that the WIS grew on a virtually ice-free area to achieve a summit thickness of 3,000 m over a period of  $\sim 25,000$  yr. This time period is in fair agreement with theoretical estimates of ice sheet growth rate<sup>21</sup>. Independent evidence<sup>16, 22</sup> suggests that the WIS extended almost to the edge of the continental shelf until  $\sim 10,000$  yr BP, when recession commenced, and ungrounding had occurred in the Ross Island area (Fig. 1) by 6,000 yr BP. Theory (R. H. Thomas, in preparation) indicates that the retreat of the WIS grounding line would proceed rather slowly until it reached Ross Island, when retreat rates would increase to  $> 1$  km yr<sup>-1</sup>. Thus WIS recession could have ceased by  $\sim 4,500$  yr BP, implying that the thinning of the ice sheet summit now observed is a delayed response to the retreat of the grounding line.

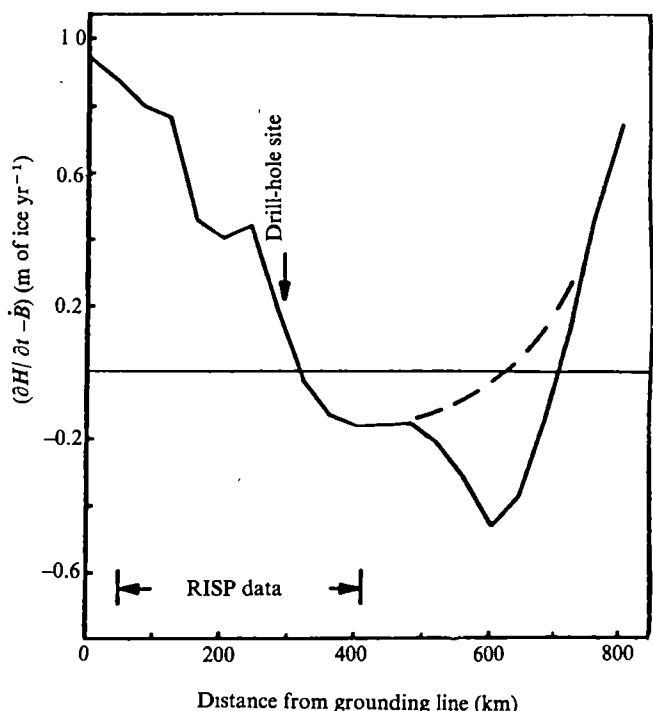
### Connection with RISP data

We must now reconcile these conclusions with the RISP observations that imply thickening at the point where ice stream 'B' enters the ice shelf. This thickening must have commenced after the period of major recession, as a consequence of either increased outflow or increased upstream constraint acting on the ice shelf near the grounding line. Increased outflow could result from upstream thinning or from enlargement of the catchment area to include that of ice stream 'C', which currently seems to be relatively inactive. We might, however, expect that present-day outflow rates are appreciably less than those during the stage of maximum recession when the ice shelf was thinning. So we shall examine the second alternative, that upstream constraints on the ice shelf increased during the past 4,500 yr

causing a decrease in the magnitude of ice shelf strain rates and, from equation (1), a positive thickening rate.

At present, the ice shelf in this area is constrained by ice rise 'P' (Fig. 1), an area where the ice shelf has grounded. Removal of the ice rise would allow more rapid ice shelf thinning and probably a grounding line recession in the south-east corner of the RIS. Thus, we can explain the present ice shelf thickening if we assume that grounding of ice rise 'P' took place comparatively recently, after isostatic uplift of the sea bed. An ice sheet completely covering the RIS would have depressed the sea bed in the area of ice rise 'P' by several hundred metres and, for a sufficiently rapid recession, much of this depression could persist until most of the present RIS had formed. Thereafter, isostatically uprising sea-bed highs could ground the ice shelf, thereby restricting continued recession of the WIS, and possibly initiating an advance of the grounding line. Evidence to support this suggestion is provided by the dimensions of ice rise 'P'. An approximate estimate of the relationship between the surface elevation and area appropriate to an ice rise can be expressed<sup>23</sup> in terms of the snow accumulation rate, the ice flow-law parameters and temperature-depth curve for the ice rise. Using current accumulation rates (H. B. Clausen and W. Dansgaard, in preparation), theoretical temperatures<sup>24</sup> and ice shelf flow-law parameters<sup>25</sup>, the equilibrium summit elevation for ice rise 'P' is found to be  $\sim 200$  m greater than observed, indicating that the ice rise is probably growing. Moreover, solution of equation (1) using data from the partially grounded ice shelf upstream of ice rise 'S' (Fig. 1) gives thickening rates up to  $0.5$  m yr<sup>-1</sup>, if we assume no bottom freezing. The ice thickness in this area is approximately equal to that of nearby freely floating ice shelf, and it seems likely that local grounding has only recently taken place. The shaded area of ice shelf in Fig. 1 indicates where the sea bed is within 50 m of the bottom of the ice shelf. If the ice shelf continues to thicken at the rates calculated above, then the WIS will advance to cover most of this

Fig. 5 A plot of  $(\partial H/\partial t - \dot{B})$  against distance from the ice shelf grounding line for the flow line that passes through the proposed RISP drill-hole site.  $\partial H/\partial t$  is the thickening rate for the ice shelf, and  $\dot{B}$  is the bottom-freezing rate. Observation errors in the RISP data limit accuracy of corresponding values of  $(\partial H/\partial t - \dot{B})$  to  $\pm 0.2$  m yr<sup>-1</sup>. Near the grounding line  $\dot{B}$  probably has a small positive value and the plot indicates a rather high rate of ice shelf thickening in this area.



area within the next 200 yr. Further advance will then be delayed by the probable lack of grounded areas in the western part of the ice shelf.

Isostatic uprise rates were at a maximum immediately following removal of the ice load, and the grounding of ice rise 'P' probably occurred shortly after the WIS recession, or ~ 4,000 yr ago. Before grounding, the local ice shelf was probably ~ 500 m thick, with its base ~ 450 m below sea level. The present bed rock high beneath the ice rise 'P' is ~ 250 m below sea level, giving an uplift of 200 m during a period of ~ 4,000 yr. Thus, the average uplift rate is ~ 0.05 m yr<sup>-1</sup> compared to a current uplift rate of 0.01 m yr<sup>-1</sup> in Fennoscandia, where the remaining uplift<sup>26</sup> is of a similar magnitude to that estimated<sup>27</sup> for the sea bed beneath the RIS. Estimates of remanent uplift are, however, subject to rather large errors, and the RIS area may be appreciably further from equilibrium than Fennoscandia. Moreover, differences in crustal thickness could result in larger uplift rates beneath the RIS.

## Conclusions

The WIS recession that began ~ 10,000 yr ago was probably initiated by the rise in sea level associated with melting of the northern ice sheets, but we should also consider the possibility of isostatic depression of the sea bed triggering recession of the grounding line. Theory<sup>20</sup> predicts that an ice sheet grounded below sea level exists in equilibrium when outflow because of snow accumulation on the ice sheet is balanced by movement of ice across the grounding line into a surrounding ice shelf. This occurs at a critical sea depth  $D$ , which is a function of ice sheet diameter, snow accumulation rate and the flow properties of ice. The ice sheet grows if sea depth is  $< D$ , and it recedes if sea depth is  $> D$ . For a growing ice sheet, isostatic depression of the bed rock continues after the grounding line has reached the appropriate critical sea depth. Moreover, crustal rigidity would cause downwarping of the sea bed for an appreciable distance to seaward of the grounding line. Consequently, sea depth at the grounding line may ultimately exceed  $D$  and trigger ice sheet recession.

If growth and decay of WIS is controlled by isostatic movements of the sea bed then we may tentatively conclude: first, that the growth rate and maximum extent of the ice sheet are determined by snow accumulation rates and sea bottom topography. Second, that the longevity of the ice sheet is determined by the rate of isostatic depression of the sea bed near the

ice sheet-ice shelf junction. Third, that after a slow start, recession proceeds very rapidly until the ice shelf formed around the shrinking ice sheet is grounded by isostatically uprising sea bed highs. Thereafter, recession is slowed and may give way to a period of ice sheet growth. Thus the minimum size of the WIS is determined by the availability of suitable obstructing ice rises and, on a sufficiently flat sea bed, almost complete disintegration of the WIS is possible. The bottom topography is, however, altered by erosion and deposition during ice sheet growth and decay so that each cycle of growth and recession follows an individual pattern that is determined by the prevailing sea bed topography. Fourth, that climatic changes probably have only a minor effect on the growth-decay sequence of the WIS. The converse may, however, not be true. In particular, complete disintegration of the WIS may alter world climate sufficiently to initiate a glacial epoch<sup>28,29</sup>.

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# Amino acids in modern and fossil woods

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*Amino acids have been found in fossil woods. The extent of amino acid racemisation increases with the age of the wood. The degree of racemisation of proline and hydroxyproline in wood from Kalambo Falls, Zambia, was used to estimate an age of >110,000 yr for the Acheulian-Sangoan transition.*

Wood is found preserved in many geological formations. Wooden tools and artefacts have also been found at many

archaeological sites. The radiocarbon dating method was first applied to a wooden artefact, an acacia wood beam recovered from the tomb of King Zoser in Sakkara, Egypt<sup>1</sup>. Radiocarbon dating however cannot be used for objects  $\gtrsim 40,000$  yr old. A wood-dating technique capable of extension beyond this present limit would be extremely useful to both anthropologists and geologists, and amino acid racemisation may provide such a technique.

The dating of fossil materials using the racemisation of amino acids is becoming well established. The kinetics and mechanism of the amino acid racemisation reaction and the use, problems and criticisms of this reaction in dating fossil materials have been discussed elsewhere<sup>2,3</sup>. The method was applied to deep-sea sediments, and fossil bones and shells<sup>2,3</sup>.

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Also, racemisation in tooth enamel has been used to assess the ages of living mammals<sup>4</sup>. These applications all involve racemisation in inorganic matrices such as calcium carbonate and calcium phosphate, but racemisation has also been shown to occur in an organic matrix, coprolites<sup>5</sup>.

Wood has an organic matrix composed mainly of cellulose, but with a complex biochemical composition because of the numerous biological processes occurring within the tree<sup>6,7</sup>. Modern woods are known to contain amino acids<sup>8-11</sup>, but there have been no investigations of the concentrations and enantiomeric composition of amino acids in fossil woods. We report here the amino acid composition and extent of racemisation in several modern and fossil woods, and a possible interpretation of these results. We emphasise that, because of the complexities and variabilities of plant biochemistry and the paucity of data on modern and fossil woods, this interpretation is preliminary.

## Method

The modern and fossil woods we have investigated are listed in Table 1. The samples were first cleaned by ultrasonication in doubly-distilled water to remove surface dirt, and were then carefully broken up into small pieces. After being rinsed in 2 N HCl, the pieces were hydrolysed in excess doubly-distilled 6 N HCl for 24 h. The hydrolysate was centrifuged to remove suspended materials, evaporated to dryness, and the residue desalted on Dowex 50W-X8 (100-200 mesh). Part of the sample was analysed on the Beckman-Spinco Model 118 Automatic Amino Acid Analyser to determine the total amino acid composition. Another fraction of the sample was analysed for enantiomeric ratios either by preparing diastereomeric dipeptides using a modification<sup>13</sup> of the method of Manning and Moore<sup>12</sup>, or by gas chromatography of the N-trifluoroacetyl (+)-2-butyl esters<sup>14</sup>.

## Total amino acid concentration

The total amino acid analyses (excluding basic amino acids) are shown in Table 1. Several interesting features are apparent. First, the total amino acid concentration per g of dry wood decreases with the age of the sample. This may arise from the hydrolysis of the bound amino acids to free amino acids (or decomposition to other organic compounds). Free amino acids, being more mobile, are later removed from the wood. A similar mechanism accounts for the loss of amino acids in fossil bones<sup>16</sup>. To demonstrate that the amino acids in fossil

woods are in the bound rather than in the free state, cleaned, sonicated wood slivers of bristlecone pine were stirred at room temperature for 60 h in 6 N HCl. After this treatment, the HCl solution was evaporated, and analysis of the remaining residue indicated the presence of few, if any, amino acids. Acid hydrolysis of the same slivers, however, yielded amino acids.

## Amino acid ratios

Another trend shown in Table 1 is the change in the relative ratios of the amino acids with time. With increasing age, the amounts of aspartic acid, threonine, and serine decreases relative to valine, while the relative amounts of isoleucine, leucine, and glutamic acid remain approximately constant. Proline is one of the most stable amino acids, and becomes predominant in the older woods. In both Kalambo Falls samples, proline constitutes ~ 25% of the total amino acid present. Kinetic studies carried out by heating samples of bristlecone pine to high temperatures, predict an amino acid stability pattern similar to that observed in the fossil woods<sup>17</sup>. This suggests that the amino acids in the fossil woods are indigenous.

The results listed in Table 1 also show that 4-hydroxyproline is present in wood. This amino acid has a fairly limited occurrence in nature and is found only in structural proteins such as collagen<sup>18</sup>. The cell walls of higher plants contain a hydroxyproline-rich glucoprotein, extensin<sup>19</sup>. This structural protein is probably the source of hydroxyproline and perhaps some of the other amino acids in wood. There is a gradual decrease in the hydroxyproline content of wood with age; a similar decrease is seen in fossil bones<sup>20</sup>.

## Enantiomeric ratios

Amino acid enantiomeric ratios for the various wood samples are shown in Table 2. In every case, the amount of racemisation increases in the order bristlecone pine < Sangoan < Acheulian. There is an unexplained discrepancy between the D/L aspartic acid ratios determined by the diastereomeric dipeptide and the gas chromatographic techniques for the Acheulian sample, but the order and the direction of increase are the same. (This is the first time we have found such a discrepancy between the two methods. This difference probably occurs because the Acheulian sample contains low quantities of the normal protein amino acids and fairly substantial amounts of unknown compounds which appear mainly in the gas chromatographic trace.) The

**Table 1** Amino acid composition of modern and fossil woods ( $\mu\text{mol per g dry wood}$ )

	Modern cherry trunk	Modern walnut root	Bristlecone pine (TRL-63-53)	Kalambo Falls Sangoan	Kalambo Falls Acheulian
Asp	1.43	1.35	0.68	0.35	0.05
Thr	1.04	0.79	0.49	0.25	0.03
Ser	1.05	1.00	0.57	0.22	0.03
Glu	1.18	0.98	0.58	0.37	0.08
Pro	0.87	0.93	0.57	0.89	0.20
OH-Pro*	—	—	0.57	0.15	0.03
Gly	1.45	1.22	0.62	0.43	0.11
Ala	1.73	1.15	0.78	0.44	0.08
Val	1.10	0.89	0.63	0.26	0.06
Ile	0.62	0.49	0.47	0.14	0.04
Leu	1.17	0.98	0.68	0.26	0.06
Tyr	0.26	0.13	0.09	0.28†	0.13†
$\phi$ -Ala	1.01	0.59	0.30	—	—
Total	12.91	10.50	6.46	3.89	0.87
Age	0	0	4,220 yr‡	> 40,000 yr§	≥ 60,000 yr

\*OH-Pro values were determined by using approximate OH-Pro/Pro values from GC analysis. Values were not determined for modern woods but the amino acid analyser results indicate OH-Pro is certainly present.

†The analyser peaks for the Sangoan and Acheulian samples were integrated by machine. Since tyrosine and phenylalanine eluted very close to one another, their integrated peak areas were not differentiated. The chromatograms show that the Tyr/ $\phi$ -Ala ratio is the reverse of that in the younger woods. This is surprising and may be due to a larger unidentified peak eluting very close to tyrosine.

‡Sample dated by dendrochronology and radiocarbon.

§UCLA-1857, Kalambo Falls, Site A, River Face (1959) Pit 3, No. 49 (9' 9"); see ref. 15.

|| Date based on stratigraphy and <sup>14</sup>C ages (J. D. Clark, Appendix J, ref. 15, and personal communication) Kalambo Falls, Site B (1959) Floors 6-8, No. 14.

Table 2 Amino acid enantiomeric ratios

	Asp	Thr	Ser	Glu	Pro*	OH-Pro*	Ala	Val	Ile	Leu	Phe
Bristlecone pine	0.11	0.016	0.022	0.065	0.049	0.031	0.044	0.020	(0.02)†	0.043	0.044
TRL-63-53	(0.09)†										
Kalambo Falls	0.13	0.016	0.026	0.13	0.28	0.057	0.10	0.052	(0.03)†	0.06	0.062
Sangoan	(0.10)†										
Kalambo Falls	0.57	(—)‡	0.078	0.48	0.60	0.101	(—)‡	0.28	(0.07)†	0.21	0.27
Acheulian	(0.21)†										

\*The D and L peaks of these two amino acids were analysed by combined gas chromatography-mass spectrometry to verify the absence of interfering peaks.

†Determined on amino acid analyser at SIO. All other values were determined by gas chromatography at NASA.

‡Not possible to determine because of interfering peaks.

increasing racemisation of the amino acids with increasing age of the wood indicates that the amino acids are indeed indigenous.

### Comparison of wood with bone and solution ratios

The relative racemisation rates of amino acids in wood seem to be different from those in bone and in aqueous solution<sup>2</sup>. The data in Table 2 indicate that proline has the fastest racemisation rate in wood. Proline also has a fast rate in aqueous solution (J.L.B., unpublished), whereas in bone its racemisation rate is comparable to that of alanine and glutamic acid (J.L.B., K. A. Kvenvolden, and E.P., unpublished). Aspartic acid, which has the fastest racemisation rate in bone and in aqueous solution, racemises at about the same rate as, or perhaps even slower than, glutamic acid in wood. Based on the Acheulian results, isoleucine epimerisation in wood seems to be slower than the racemisation of valine and leucine. All three of these amino acids have nearly the same racemisation rates in bone and in aqueous solution. The gas chromatogram of the Acheulian sample showed numerous unidentified peaks eluting near valine, alanine, leucine and serine, and it is possible that spurious peaks caused erroneous D/L ratios for these amino acids.

Based on analyses of bones from sites in east and south Africa, racemisation in wood seems to be much slower than in bone<sup>21,22</sup>, and to confirm this, high temperature kinetics experiments were carried out. Bristlecone pine samples (TRL-71-3, ~ 3,170 yr) were used in these studies, rather than modern wood. Modern woods behaved very differently during these experiments, and had high D/L aspartic acid ratios (0.16 and 0.12). Modern wood tended to stain glassware and smoke profusely during acid hydrolysis, whereas the older woods did not (probably because of the absence of sap). The sap of modern woods has been shown to contain free amino acids<sup>10</sup>, whereas free amino acids are absent in the bristlecone pine sample. The bristlecone pine behaved more like the fossil woods during these experiments. Apparently the organic substances present in the wood sap are labile and have decomposed or have diffused out of the wood after 3,000 yr.

The kinetics experiments run on bristlecone pine (TRL-71-3) showed that the epimerisation of isoleucine follows reversible first-order kinetics<sup>17</sup>. The first-order rate for conversion of isoleucine to alloisoleucine,  $k_{iso}$ , was determined at several temperatures. These results, along with the  $k_{iso}$  values in aqueous solution<sup>23</sup> and bone<sup>24</sup>, are shown in Fig. 1. The high temperature results confirm that the racemisation rates in wood are much slower than those in bone or in aqueous solution ( $k_{iso, bone} \approx 10 \times k_{iso, wood}$ ). The Arrhenius activation energy in wood, 29.4 kcalorie mol<sup>-1</sup>, is similar but slightly lower than in bone ( $E_a = 33.3$  kcalorie mol<sup>-1</sup>) or in aqueous solution ( $E_a = 31.3$  kcalorie mol<sup>-1</sup>).

### Interpretation

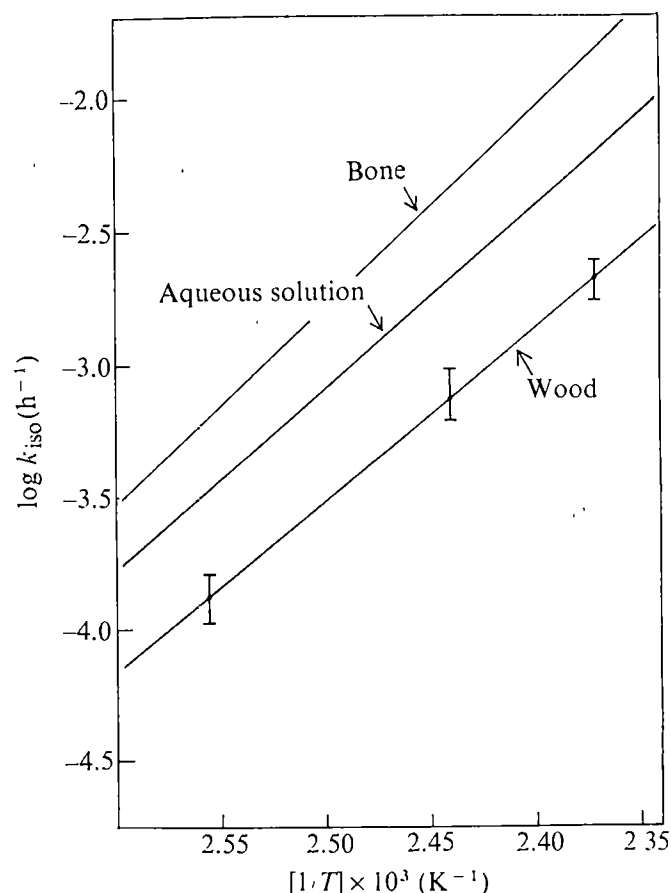
The slower racemisation rate in wood may occur because the amino acids in wood are bound in an organic matrix. Since racemisation requires the presence of water<sup>20,21</sup>, a hydrophobic environment in wood could slow down the racemisation rate—

bones impregnated with tar have racemisation rates considerably slower than bones in normal geological environments<sup>16</sup>. Racemisation studies in coprolites, also an organic matrix, similarly show rates of racemisation slower than bones from the same location (J.L.B., and M. G. Petit, unpublished). One important difference between the isoleucine epimerisation kinetics in wood and those of other systems, is that the equilibrium allo/iso ratio is 0.86 in wood, compared with 1.25–1.40 for aqueous solution and bone<sup>20,21</sup>. Again, this difference may arise from the organic nature of the wood.

### Ages

The racemisation results we have obtained for the Kalambo Falls woods can be used to calculate an age for the upper Acheulian industry at this site. The racemisation dating technique is best applied by 'calibrating' the rate of the amino acid racemisation reaction for a particular site using the extent of racemisation in a sample which has been radiocarbon dated. After this 'calibration' has been carried out, other samples from the same site can be dated based on the extent of their

Fig. 1 Arrhenius plot of isoleucine-alloisoleucine epimerisation kinetics in wood, in aqueous solution<sup>23</sup> and in bone<sup>24</sup>.



racemisation. The ages of fossil bones deduced using this technique are in close agreement with radiocarbon ages and the ages deduced from other evidence<sup>21,22</sup>.

Our Sangoan sample was radiocarbon dated at > 40,000 yr old. Therefore, by using the Sangoan sample to calibrate the racemisation rate in wood at Kalambo Falls, only a minimum age can be assigned to the Acheulian sample. Calculations of this minimum age can be made using the enantiomeric ratios from each of the amino acids appearing in Table 2. Certain amino acids, however, are better than others. Aspartic acid is unsuitable because of the discrepancy between the D/L ratios determined using the gas chromatographic and diastereomeric dipeptide techniques. Because of the very small extent of racemisation of valine, leucine, phenylalanine and isoleucine in the Sangoan sample, it is difficult to calculate accurate calibration rate constants. The best choices for the calibration procedure are proline and hydroxyproline. Proline is the most stable amino acid in wood, and is still present in sizeable amounts in older woods (see Table 1). Proline also has the fastest racemisation rate, according to our preliminary data. Hydroxyproline is also a good choice for use in the calibration method because it has a much different (10 times slower) rate of racemisation (epimerisation) than proline, and it is not subject to contamination problems, because of its limited occurrence in nature.

Amino acid racemisation ages and the calibration rate constants are calculated from the following equation<sup>2</sup>

$$\ln \left\{ \frac{1 + (D/L)}{1 - K'(D/L)} \right\} \bigg|_{\text{sample}} - \ln \left\{ \frac{1 + (D/L)}{1 - K'(D/L)} \right\} \bigg|_{t=0} = (1 + K')kt$$

where  $k$  is the first-order rate constant for interconversion of the D- and L-enantiomers of a particular amino acid and  $K'$  is the inverse of the equilibrium constant  $K_{eq}$ , which equals the D/L ratio at equilibrium<sup>2</sup>. For amino acids with more than one centre of asymmetry, such as hydroxyproline,  $K_{eq}$  is slightly different from unity. Experimental determinations of  $K_{eq}$  for hydroxyproline show that the equilibrium constant in aqueous solution is 0.8 (ref. 20). Since hydroxyproline epimerisation in the fossil wood samples we have analysed, is so far from equilibrium, the value of  $K_{eq}$  makes little difference to our calculations. Using the D/L-proline and allohydroxyproline/hydroxyproline ratios determined for the Sangoan wood sample, an age of > 40,000 yr for the Sangoan, and the bristlecone pine at  $t = 0$  values, the calculated racemisation rates are  $k_{OH-P_{ro}} \leq 6.5 \times 10^{-7} \text{yr}^{-1}$  and  $k_{P_{ro}} \leq 6.0 \times 10^{-6} \text{yr}^{-1}$ . These values of  $k$ , combined with the D/L proline and allohydroxyproline/hydroxyproline ratios for the Acheulian wood, give ages of > 110,000 yr for hydroxyproline and > 110,000 yr for proline. The agreement of these two values is excellent,

especially considering the order-of-magnitude difference in rate constants. It should be pointed out that similar calculations using the other amino acids listed in Table 2 all give older minimum ages. The ages deduced from the other amino acids are less reliable, however, for the reasons we have discussed earlier. In any case, the combined racemisation results show that > 110,000 yr is an absolutely minimum age for the Sangoan-Acheulian transition at Kalambo Falls.

The age of the end of the Acheulian industry in Africa has sometimes been used to define the beginning of the Upper Pleistocene, a time span poorly defined by geochronometric means<sup>26</sup>. (The Upper Pleistocene is more usually defined as coincident with the beginning of the Last (Eemian) Interglacial at ~ 125,000 yr (ref. 26).) Our racemisation-deduced age of > 110,000 yr at Kalambo Falls is consistent with numerous radiometric dates for older Acheulian sites in Africa<sup>25</sup>, and compares well with geologically-inferred dates for the beginning of the Eemian and the end of Acheulian industry in southern Africa<sup>27</sup>.

We thank J. Desmond Clark for suggesting our work on the Kalambo Falls site, for providing us with samples, and for his comments on the manuscript. We also thank Rainer Berger for radiocarbon dating of the Sangoan wood, Hans Suess and Tim Linick for providing us with the samples of bristlecone pine, and John Turk for the modern wood samples. This research was supported by the NSF.

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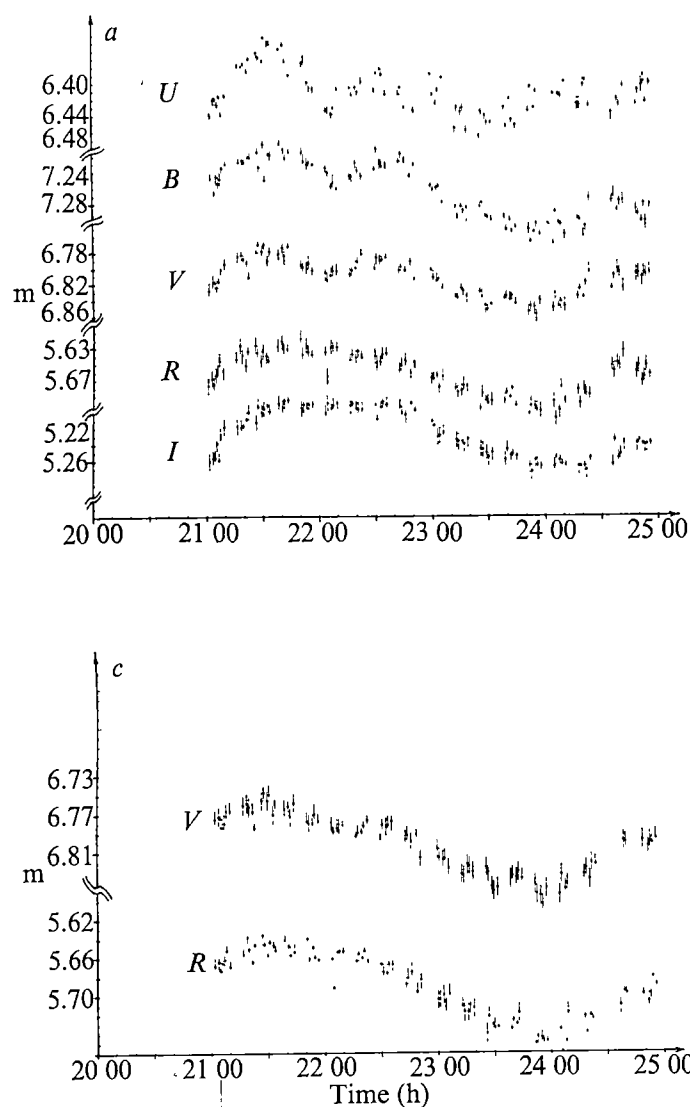
## letters to nature

### UBVRI photometry of Nova Cygni 1975

MULTICOLOUR photometric observations have been made of Nova Cygni 1975. A periodic variation of ~3.2 h was detected by Tempesti<sup>1</sup> on 1975 September 14 when the nova was at  $m_v = 6$ . On 1975 September 18 we made a 4-h survey in five colours, UBVRI, to check this variation using the 40-cm telescope of the Laboratorio di Astrofisica Spaziale CNR at Frascati, in the Monte Porzio Observatory. The

variation was confirmed for all colours, and other significant characteristics of the light curve have been detected.

The photoelectric photometer used, the DAPHNE system, has been described elsewhere<sup>2</sup>; its main features are: the photomultiplier used is the RCA C31034A in photon counting mode, with the GaAs photocathode cooled to -20 °C, measurements in the five bands are serial with the possibility of choice of acquisition time; and each sequence of measurements can be started with a quartz clock at a well



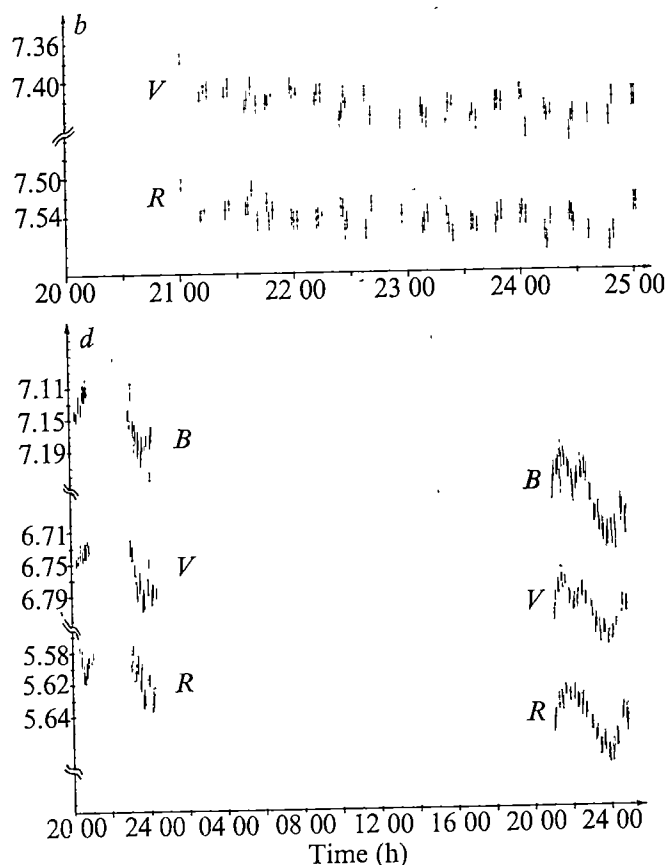
defined time. An electronic offset device switches the telescope from the comparison star to the nova, and vice versa; so that data from the comparison star and nova can be obtained with a minimal interval. The acquisition time was 3 s for each band, and the magnitudes in the five colours have been plotted and are shown in Fig. 1a.

All the light curves show a variation  $\sim 0.08$  mag. Moreover, a new feature is present in every band: a secondary minimum appears centred at 22 h 05 min, shifted  $\sim 110$  min from the maximum. The relative intensity of this anomalous absorption varies with colour, vanishing towards the long wavelength end.

Measurements of the comparison star, BDA473294, with respect to a set of standard stars on September 18 are shown in Fig. 1b. The error bar of each measurement ( $\pm 0.01$  mag r.m.s.) was derived from a check on standard stars. The magnitudes in the five bands derived for the comparison star are:  $U=7.49$ ,  $B=7.48$ ,  $V=7.43$ ,  $R=7.54$ ,  $I=7.69$ , which have been used as a standard for computing the nova magnitudes. No periodic variation was detected, in either magnitude or colour for the comparison star. The same variation of luminosity of the nova is still obtained after applying the coefficients for the night obtained from standard star measurements (see Fig. 1c). This excludes any possibility of intrinsic variation in the comparison star and confirms the short time variation of the nova.

Figure 1d shows the results for September 17 and 18 together. The decrease of the mean intensity is evident, with a rate of almost  $0.1 \text{ mag d}^{-1}$ . Any period from 3 h 00 min to 3 h 48 min fits the two sets of measurements:

Fig. 1 a, Five colour, 4-h photometry of Nova Cygni 1975, on September 18, 1975. b, V and R photometry of the comparison star, obtained from standard stars. c, V and R photometry of the nova, obtained from standard stars. d, B, V, R photometry of the nova for the nights of September 17 and 18.



this implies either different features from night to night, or a real period  $> 3 \text{ h } 48 \text{ min}$ .

We are grateful to Professor P. Tempesti for prompt communication on this atypical feature of the nova.

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## Coronal lines in near infrared spectrum of Nova Cygni 1975

WE have been monitoring the 2- and 3- $\mu\text{m}$  spectral regions of Nova Cygni 1975 at irregular intervals. Shortly after maximum, these spectral regions were dominated by the lines of hydrogen and a few of neutral helium superposed on a continuum presumably of free-free radiation. These spectra could be quantitatively interpreted on traditional recombination theory<sup>1</sup>, assuming a temperature of  $\sim 10^4$  K. Beginning with a spectrum



obtained on September 29, 1975 a number of additional lines have appeared in the spectrum. These new lines have increased in strength so that in our most recent data they nearly dominate these spectral regions. These two phases of the spectral development of Nova Cygni 1975 are illustrated for the 2- and 3- $\mu$ m spectral regions in Figs 1 and 2.

To isolate more clearly the additional spectral features, we have subtracted the earlier data, suitably scaled, from the most recent spectra. These difference spectra, also displayed in Figs 1 and 2, demonstrate that the spectral changes result from additional lines superposed on a nearly-unaltered recombination spectrum.

The derived wavelengths and proposed identifications for these new lines are listed in Table 1. The near infrared spectrum of Nova Cyg 1975 is now dominated by forbidden lines arising from highly ionised species of Mg, Al, and Si. These lines are analogous to the coronal lines observed in the visible portion of the solar spectrum. One of them, the [Mg VIII] line at 3.03  $\mu$ m, has been previously observed in the solar corona<sup>2,3</sup>. Although at first sight outlandish, these identifications appear entirely secure. None of the wavelength differences is larger than might be expected on the basis of our error in measuring the wavelength and the expected uncertainties in the predicted wave-

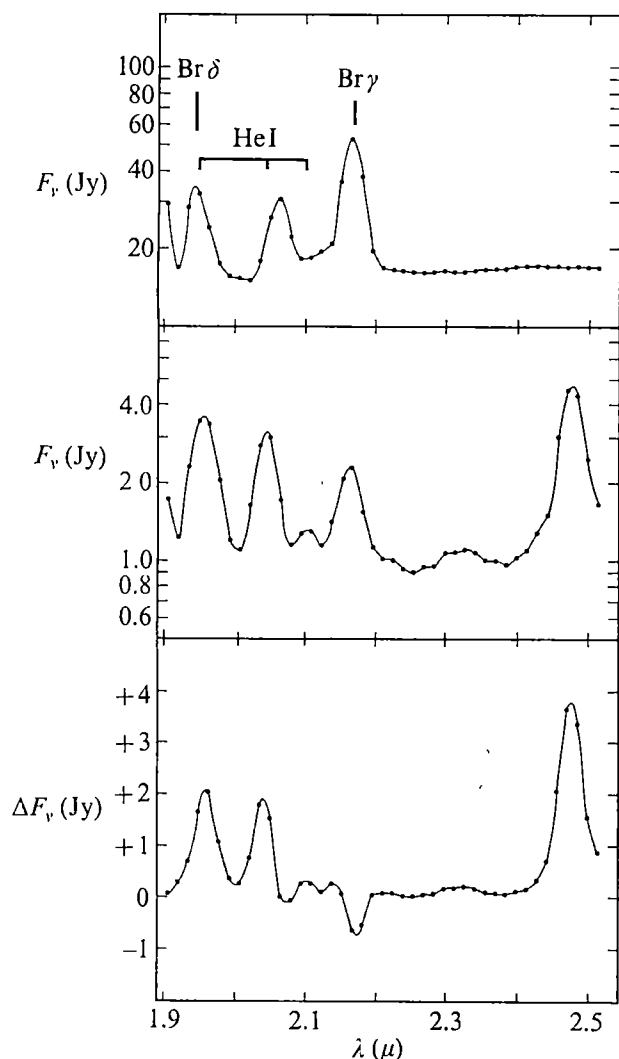


Fig. 1 The 1.9–2.5  $\mu$ m spectrum of Nova Cygni 1975 observed with an InSb detector and a circular variable interference filter having a resolution of 1.3%. The coronal lines are unresolved at this resolution. The difference spectrum in the lower panel results from subtraction of the 1975 September 12 data (top) divided by 18, from the 1975 October 27 spectrum (middle). The emission features near 2.10 and 2.14  $\mu$ m are probably artefacts of this procedure.

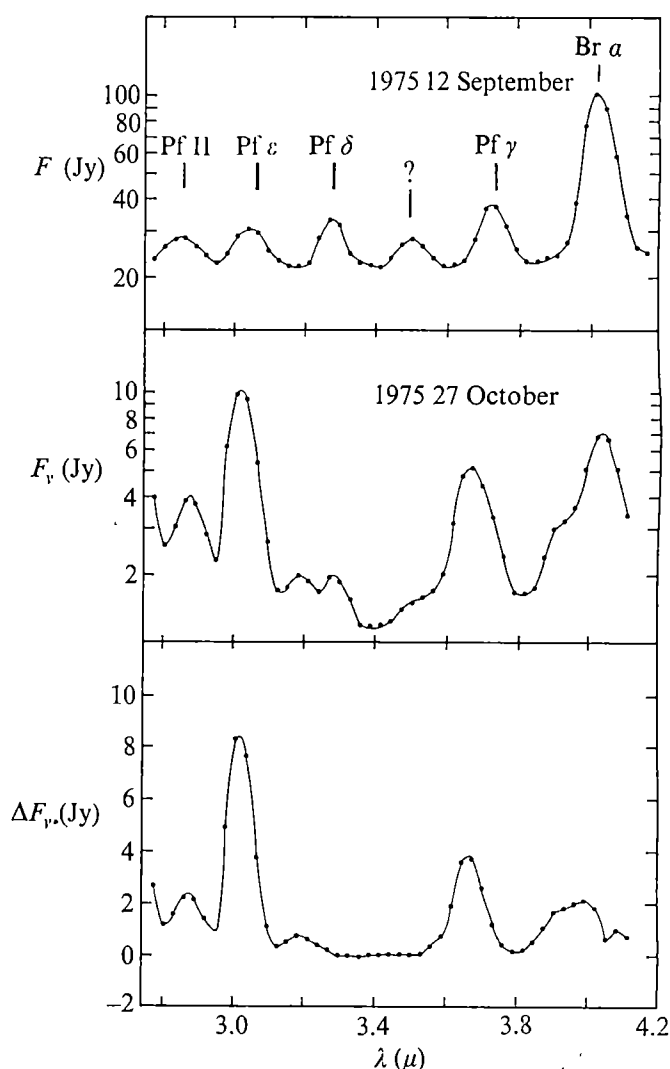


Fig. 2 The 2.8–4.1  $\mu$ m spectrum of Nova Cygni 1975 observed with an InSb detector and a circular variable interference filter having a resolution of 2.2% on the same dates as in Fig. 1. The difference spectrum was obtained in the same manner as that in Fig. 1.

lengths. Even more convincing is the fact that virtually all the coronal lines expected in these portions of the spectrum are present. Considering  $\Delta J = 1$  transitions within the ground state of ions of elements whose normal abundance is  $> 10^{-6}$  that of hydrogen, only two predicted lines are not definitely present. One, the line at 4.09  $\mu$ m for [Ca VII], is inextricably blended with Brackett  $\alpha$  at 4.05  $\mu$ m. The other, at 2.29  $\mu$ m, would come from [Ca XIII] whose ionisation potential of 726 eV adequately explains its absence.

This result has a number of interesting consequences. Since these coronal lines have been increasing in absolute strength, a region with very high temperature,  $\sim 10^6$  K, must be developing within the nova material. Furthermore, our data indicate that the degree of ionisation is still increasing, as the [Al IX] and [Si IX] lines have been increasing more rapidly than the other coronal lines. The visible spectrum of Nova Cyg 1975 does not yet, however, exhibit any coronal lines (J. Cohen, personal communication) although coronal lines have been observed in a number of previous novae<sup>4</sup>. Apparently, for reasons that are not yet clear, the near infrared provides a more sensitive indicator of the presence of a coronal zone than does the visible spectrum. This raises the possibility that the development of a very high temperature zone in novae is more common than presently deduced from visible data alone.

Table 1 Coronal line identifications in Nova Cygni 1975

$\lambda_{\text{obs}}(\mu\text{m})$	$\sigma$	$\lambda_{\text{pred}}(\mu\text{m})$	Identification		
1.959	0.007	1.963 (ref. 6)	[Si VI]	$^2P_0$	(1/2, 3/2)
2.040	0.007	2.045 (ref. 6)	[Al IX]	$^2P_0$	(3/2, 1/2)
2.32	0.02	2.323 (ref. 7)	[Ca VIII]	$^2P_0$	(3/2, 1/2)
2.474	0.007	2.481 (ref. 5)	[Si VII]	$^3P$	(1, 2)
2.879	0.014	2.904 (ref. 6)	[Al V]	$^2P_0$	(1/2, 3/2)
3.021	0.014	3.030 (ref. 6)	[Mg VIII]	$^2P_0$	(3/2, 1/2)
3.18	0.03	3.210 (ref. 8)	[Ca IV]	$^2P_0$	(1/2, 3/2)
3.661	0.014	3.656 (ref. 5)	[Al VI]	$^3P$	(1, 2)
3.72	0.02	3.700 (ref. 5)	[Al VIII]	$^3P$	(2, 1)
3.92	0.02	3.917 (ref. 5)	[Si IX]	$^3P$	(1, 0)

Finally, since a number of ionisation stages are observable for Si and Al, we may be able ultimately to deduce the physical conditions within the coronal zone. This raises the exciting prospect of being able to deduce relative elemental abundances for the high temperature component. One might expect the high temperature zone to be more intimately involved with the source of the explosion, and thus its chemical composition may contain important clues for our understanding of the nova process.

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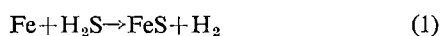
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## Formation of iron sulphide in solar nebula

IODINE–XENON dating has established that iron sulphide in the Orgueil carbonaceous meteorite is one of the oldest known meteoritic mineral phases, and probably dates from the condensation stage of the early Solar System<sup>1</sup>. This sulphide, although generally assumed to be troilite, FeS, is in fact the Fe-deficient monosulphide, pyrrhotite,  $(\text{Fe,Ni})_9\text{S}_{10}$ , containing ~1 weight % Ni (ref. 2). The purpose of this note is to suggest that such mineral chemistry is inconsistent with equilibrium condensation, and that the course of condensation may have been modified by kinetic effects.

Strictly, iron sulphide is not a condensate but a reaction product between a condensate, metallic Fe, and S in the gas phase. The reaction

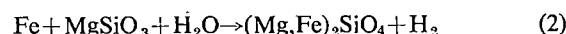


goes to the right at 680 K, in a cooling nebula of solar composition<sup>3</sup>. It is necessary to consider the effect of Ni on this reaction and the course of equilibrium condensation may be determined by means of the Fe–Ni–S ternary phase diagram (Fig. 1). This shows that the solar composition lies within the kamacite–taenite–troilite stability field at 680 K. Thus, formation of troilite, which can contain no more than 0.5 weight % Ni (ref. 4), from kamacite containing 6 weight % Ni would require precipitation of taenite, with ~40 weight % Ni (ref. 4). Consequently, with the

commonly accepted value of 1.7 for the solar Fe/S ratio<sup>6</sup>, the only iron sulphide predicted to exist at equilibrium is stoichiometric troilite, FeS, containing <0.5 weight % Ni. It is therefore pertinent to ask why the Orgueil sulphide differs in both crystal structure and Ni content from the predictions of equilibrium condensation. Four possible reasons may be advanced.

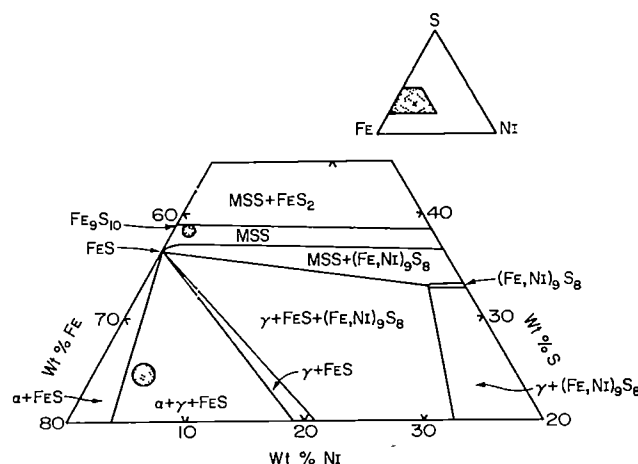
First, the value taken for the solar Fe/S ratio may be in error. A value of <1 could result in pyrrhotite being a stable phase. Depending on the actual value of Fe/S, this pyrrhotite would either be accompanied by another sulphide (either pentlandite or pyrite) or fall compositionally within the monosulphide solid solution field. In this last case, the composition of the mineral would be exactly equal to the solar proportions of the three elements. Unless the value taken for the solar Fe/Ni ratio is also in error, in the opposite sense, such is not the case for Orgueil pyrrhotite (Fig. 1) nor does the meteorite contain any pentlandite or pyrite.

Second, stabilisation of another Fe-bearing phase could result in competition for the available Fe and consequent formation of an Fe-deficient sulphide. At around 680 K the only plausible alternative is production of fayalitic olivine from enstatite



At this temperature, however, the maximum possible FeO content of olivine in equilibrium with the nebular gas is ~5 weight %, which is inadequate to alter the course of sulphide condensation. In addition, this reaction is between two solid phases and is unlikely to compete effectively with the gas–

Fig. 1 Phase relations in a portion of the Fe–Ni–S system in equilibrium with vapour at 680 K and  $\leq 1$  atm pressure, based on refs 4, 5. Large circle represents solar composition<sup>6</sup>, small circle shows composition of Orgueil pyrrhotite<sup>2</sup>. MSS is monosulphide solid solution, FeS<sub>2</sub> is pyrite; FeS is troilite,  $(\text{Fe,Ni})_9\text{S}_8$  is pentlandite,  $\alpha$  is kamacite, body-centred cubic Fe;  $\gamma$  is taenite, face-centred cubic Fe



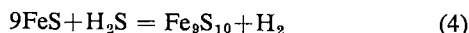
solid reaction. It would also result in a sulphide with a Ni/Fe ratio greater than solar, rather than less, because meteoritic olivines contain only trivial amounts of Ni.

A third possibility is that oxidation<sup>8</sup> of a Ni-poor troilite condensate might have produced the observed Ni-bearing pyrrhotite, if the Ni was retained in a sulphide phase comprising a small fraction of the original S. Several reaction pathways are possible; the observed Orgueil mineralogy (pyrrhotite plus magnetite plus epsomite, MgSO<sub>4</sub>) suggests the following representation



It is far from certain, however, that such a reaction could have occurred. If it did occur it must have been after accretion of the meteoritic parent body to retain the oxidised species of sulphur. Therefore the <sup>129</sup>I/<sup>127</sup>I ratio inferred by Lewis and Anders<sup>1</sup> would have been characteristic of some late stage in planetary accretion rather than of the condensation stage, as they propose. Such a conclusion would impose severe constraints on models of early Solar System accretion, especially as accumulation of the other meteorite parent bodies apparently took place after the Orgueil alteration stage<sup>1</sup>. Another argument against the oxidation hypothesis is that pyrrhotite-magnetite associations are virtually never found in Orgueil. Where alteration of pyrrhotite is found, the reaction products are sulphate, elemental sulphur and limonite (an hydrated iron oxide of variable composition).

A more reasonable possibility is that sulphide formation in the nebula was inhibited by sluggish diffusion, so that sulphur began to react with previously condensed troilite to form pyrrhotite



This reaction is also capable of explaining qualitatively the Ni content of Orgueil pyrrhotite. Initial formation of troilite would have resulted in the enrichment of Ni in the metal at the interface with the growing sulphide<sup>4</sup>. Conversion of this sulphide to pyrrhotite, which can accept large amounts of Ni and in which diffusion is likely to be easier than in either troilite or kamacite, would result in transfer of some Ni from metal into sulphide. The amount of such Ni would presumably have been kinetically limited.

Observations on Orgueil sulphides therefore suggest that below ~700 K the course of solar system condensation was modified by kinetic effects and that equilibrium may not have been achieved. These conclusions have implications for the Bi, Tl, In cosmo-thermometers which are considered to be applicable over the temperature range 420–500 K (refs 9, 10). In fact, the reactions considered here are particularly appropriate because Bi and Tl are believed to condense as alloys in metallic Fe, and In as a sulphide in troilite. If sulphur failed to reach equilibrium with Fe at 680 K, it is difficult to understand how Bi and Tl could have done so at a temperature 200 K below. Recall that, by then, all available Fe would be covered with a layer of pyrrhotite. A final detail is that the solubility data used in the thermodynamic calculations, on which the equilibrium theories are based, were for troilite rather than pyrrhotite, in which heavy trace elements are probably more soluble than in stoichiometric troilite.

Identification of Orgueil pyrrhotite as a nebular condensate is also in conflict with predictions of Blander's Constrained Equilibrium Theory<sup>11</sup>, where, as a result of inhibited nucleation of metallic Fe and FeS, troilite is predicted to condense at temperatures in the range 1,050–1,450 K. At such high temperatures it is unlikely that reaction (1), once nucleated, would fail to reach equilibrium.

These conclusions apply strictly only at the formation location of the carbonaceous meteorites. In the absence of evidence to the contrary, such as, hypothetically, occurrence of primordial troilite in some other type of meteorite, they may be applied to condensation at any point in the Solar System swept by the 680 K isotherm.

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## Atmospheric shock waves and condensation clouds from Ngauruhoe explosive eruptions

VISIBLE shock waves ('flashing arcs') passing through the air above a volcanic vent were first recognised by Perret<sup>1</sup> during explosive eruptions of Vesuvius in 1906. Although reportedly rare<sup>2,3</sup> in subsequent volcanic eruptions, large visible atmospheric shock waves have accompanied explosive activity of Ngauruhoe, an andesite volcano in New Zealand, in 1954 (ref. 4), 1974 (ref. 5) and 1975.

During a series of seven highly explosive eruptions on the afternoon of February 19, 1975, observational and photographic records of eruption sequences were obtained, the most complete being of the largest explosion at 1810 NZDT which accompanied a volcanic earthquake of magnitude *M<sub>L</sub>* 3.4 (J. H. Latter, personal communication). Observations were made from both Mangatepopo Track and Chateau Observatory, respectively 3.4 km and 9.2 km from, and 1,110 m and 1,170 m below, the 100-m deep funnel-shaped summit crater. Each eruption was an isolated event following a 20–60-min period during which ash emission and volcano-seismic activity declined to very low levels. Explosion initiation appeared instantaneous, heralded by a large atmospheric shock wave clearly visible as a luminous arc expanding very rapidly through thin cloud, steam and clear air to several hundred metres above the crater. Attempts to photograph the shock waves were unsuccessful. Each shock wave was closely followed by the cannon-like, high-velocity projection above the crater of a dense slug of highly compressed gas and solid ejecta. Apparently due to vent shape the eruption slug formed three expanding sub-cylindrical coalescing plumes with rounded outlines and slightly pointed tops (Fig. 1a), when 200 m above the crater rim. The first photograph of the explosion was taken at this instant, between 0.5 and 1.0 s after observation of the shock wave. Figure 1b, taken by another observer a fraction of a second after Fig. 1a, records development of a sub-spherical hollow 'condensation cloud', surrounding and partly obscuring the expanding eruption slug. The diameter of the condensation cloud at this moment was ~500 m, although it extended a greater distance above the crater than down the slopes of the volcano (by ~1.8:1). Figure 1c, taken ~1 s later by a third observer, records disappearance of the condensation cloud, and outward expansion of the eruption slug to envelop the summit, while large blocks (up to 20 m across) were projected through it. Eight-millimetre cine film (18 frames s<sup>-1</sup>) taken of the explosion between photographs 1b and c, records the base of the condensation cloud 170 m above the crater (first frame), with its top extending into atmospheric cloud 580 m above the crater. The base of the condensation cloud travelled upwards at about 190 m s<sup>-1</sup> to reach 220 m above the crater

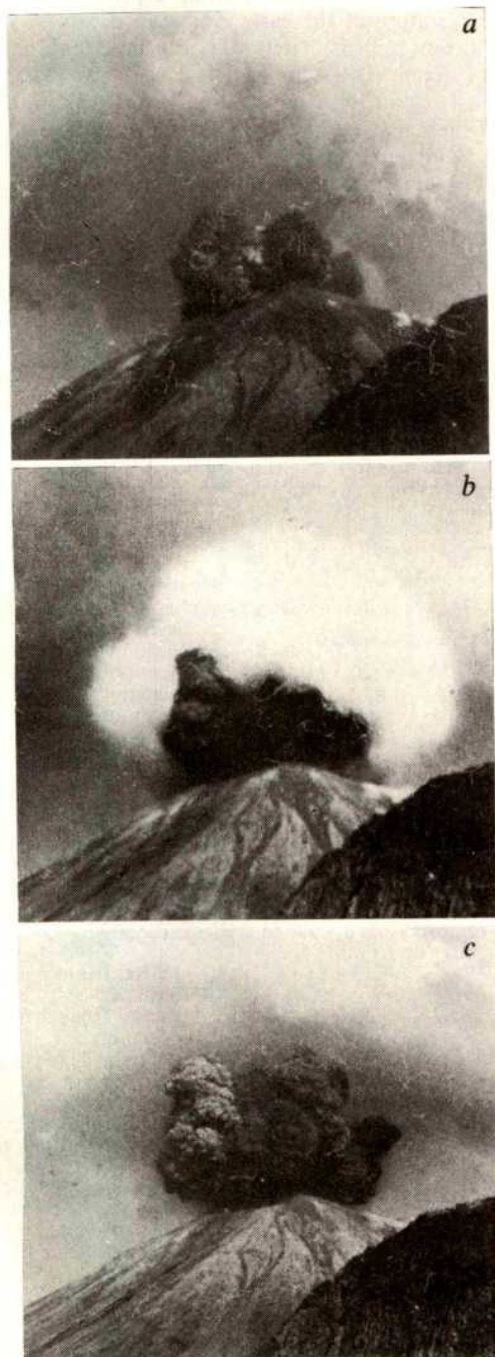


in the sixth frame of the film, before disappearing entirely in the seventh frame, exposed  $\sim 0.4$  s after the first.

The audible shock wave reached the Mangatepopo observation point as a very sharp and intensely loud 'crack' arriving 10.6 s after observation of the visible shock wave (G. T. Hancox, personal communication). This is equal within limits of error to the theoretical sound travel time of 10.58 s (assuming a sound velocity of  $338 \text{ m s}^{-1}$  in still air at  $10^\circ\text{C}$ ).

The atmospheric shock waves from each explosion were recorded as air-ground coupled waves at the Chateau seismograph 8.6 km from the crater. Air shock waves were also recorded on a nearby barograph. Each explosive pulse (up to

**Fig. 1** Sequential photographs of the 1810 NZDT eruption, February 19, 1975, taken by three observers reacting to the appearance of the preceding shock wave. *a*, Eruption slug projected above crater; *b*, expanding eruption slug surrounded by 'condensation cloud'; *c*, disappearance of condensation cloud. Photographs from Mangatepopo Track by G. T. Hancox, D. M. McVicker, and R. Foley.



$2.5 \times 10^2 \text{ Pa}$  above ambient in the 1810 eruption) was accompanied by a lesser fall (to  $1.5 \times 10^2 \text{ Pa}$  below ambient) in the following rarefaction phase. The explosions were heard more than 80 km from the volcano.

Short lived condensation clouds similar to those which formed at Ngauruhoe accompany nuclear explosions, an example being particularly clearly illustrated in photographs of the 1946 Bikini underwater explosion<sup>6,7</sup>. Formation of the cloud is due to rapid condensation of atmospheric water vapour in the adiabatically cooled rarefaction phase which is developed behind the positive overpressure phase of the shock wave. Subsequent re-evaporation and disappearance of the cloud occurs as atmospheric pressure returns to ambient (and temperature to slightly above ambient) behind the rarefaction phase<sup>6</sup>. The development of such condensation clouds does not appear to have been previously recorded in volcanic explosions. They were not recognised by scientific observers during the Ngauruhoe eruptions, and their existence was only realised after study of the photographs.

The shape of the 1810 NZDT condensation cloud (Fig. 1*b*) indicates that the initial near-planar shock wave within the vent had diffracted around the crater rim to produce a subspherical blast wave. The departure from sphericity of the cloud also suggests that if the shock moving down the cone had slowed to sonic speed (as seems likely from crater geometry and the measured sound travel time) the shock velocity directly above the crater was at least 1.8 times greater at Mach number  $M = 1.8$ , or  $\sim 600 \text{ m s}^{-1}$ .

An estimation of the initial velocity of the 1810 NZDT ejecta slug is hindered by lack of accurate timing of the first photographs, the limits of 0.5–1.0 s after explosion initiation indicating an average ejecta slug velocity ranging between 300 and  $600 \text{ m s}^{-1}$  over the first 300 m. Several methods of determining initial ballistic ejecta velocities from maximum range within the atmosphere have been developed, most recently by Wilson<sup>8</sup> who allowed for an increased drag coefficient ( $C_d$ ) at high Mach numbers. During the Ngauruhoe eruptions, a 0.8 m diameter lava block of density  $2.5 \text{ g cm}^{-3}$  was thrown 2.8 km, landing 980 m below the vent. Assuming a horizontal range of 2.5 km, a minimum initial velocity approaching  $400 \text{ m s}^{-1}$  is interpolated from Wilson's tables. That this is of the correct order may be indicated by the relationship from one-dimensional shock tube theory<sup>9</sup>

$$U = \frac{u_p}{2-2\mu^2} + \left( c^2 + \frac{1}{4} \left( \frac{u_p}{1-\mu^2} \right)^2 \right)^{1/2}$$

where  $U$  = shock velocity,  $u_p$  = piston velocity,  $c$  is velocity of sound, and  $\mu^2 = 1/6$  for air. A minimum initial Ngauruhoe shock velocity of  $600 \text{ m s}^{-1}$  (as suggested by condensation cloud shape) would be associated with a piston (that is, ejecta slug) minimum velocity of about  $340 \text{ m s}^{-1}$ . Similar velocities ( $> 330 \text{ m s}^{-1}$ ) have been observed for venting gases and ejecta in the Sedan underground thermonuclear explosion<sup>10</sup>, and considerably higher velocities ( $600\text{--}660 \text{ m s}^{-1}$ ) have been calculated for ejecta from explosive eruptions of Arenal, a volcano in Costa Rica<sup>8,11</sup>.

For cannon-like volcanic explosions, McBirney<sup>12</sup> has derived the equation  $P = 0.0125v^2$ , where  $P$  is the explosion pressure (atm) and  $v$  is ejecta velocity ( $\text{m s}^{-1}$ ). Assuming an initial ejecta velocity of  $400 \text{ m s}^{-1}$ , a minimum explosion pressure of  $2 \times 10^8 \text{ Pa}$  is calculated for Ngauruhoe. McBirney also deduces that such high pressures cannot be generated by the exsolution of volatiles from magmas of normal water content, but, instead, are caused by the rapid heating of confined meteoric water to near magmatic temperatures. This model it appears can be applied to Ngauruhoe, where a lava column rising within the volcano may rapidly heat pockets of meteoric water confined beneath the solid lava of an earlier formed plug<sup>5</sup>. High pressures develop until the mechanical



strength of the confining rocks is exceeded, and catastrophic failure occurs. Intense steam explosions result, with mechanism perhaps similar to that envisaged by Bennet<sup>13</sup>. At Ngauruhoe, the almost instantaneous release of pressures apparently  $> 2 \times 10^8$  Pa, at supersonic velocities, is clearly adequate to generate large visible shock fronts above the volcano.

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## Temperature limits on the early Archaean ocean from oxygen isotope variations in the Isua supracrustal sequence, West Greenland

FROM a reconnaissance survey of the oxygen isotope composition of a sequence of metamorphosed sedimentary rock units at Isua, West Greenland, it is possible to set wide temperature limits of  $\sim 0$ – $146^\circ\text{C}$  on the early Archaean ocean.

The study area, part of the Greenland Archaean terrain, is 150 km north-east of Godthaab. The Isua supracrustal sequence, which consists of conglomerate, quartzite, acid metavolcanics, garnet-staurolite schist, banded iron formation, and laminated chert and carbonate interlayered with mafic flows or sills, may have been deposited in shallow water<sup>1</sup>. This sequence is enclosed by gneisses (Fig. 1) that range in composition from granodiorite to granite, and both the supracrustal sequence and gneissic terrain have been metamorphosed to amphibolite facies. A Pb–Pb age for the supracrustal sequence of  $3,760 \pm 70$  Myr has been determined by Moorbath *et al.*<sup>2</sup> from whole rock and from mineral separates of the banded iron-formation while an age of  $3,700 \pm 100$  Myr has been reported<sup>3</sup> for the gneissic terrain.

Oxygen isotope analyses were performed on laminated and massive cherts and on quartz separates from banded iron formations. Oxygen was liberated from 10–20-mg samples by bromine pentafluoride and converted to  $\text{CO}_2$  for mass spectrometric analysis<sup>4</sup>. Analyses were performed on a 15-cm,  $60^\circ$  sector mass spectrometer with a double inlet and a double collector.

The  $\delta^{18}\text{O}_{\text{SMOW}}$  values<sup>5</sup> for samples from three areas at Isua are presented in Table 1. Area one is the main iron formation occurrence at Isua<sup>2,6</sup>.  $\delta$ -values of laminated and massive chert units range from  $+16.1$ – $+20.3\text{‰}$ , the latter being the largest  $\delta$ -value reported for a precipitated Archaean siliceous sediment. The massive chert units in the area of the main iron-formation occurrence at Isua seem to have undergone limited rehomogenisation; therefore, the ratios may closely approximate the initial ratio of these units. Quartz separates from the banded iron formations in Area One range from  $+12.6$ – $+15.9\text{‰}$ , showing a probable re-equilibration of the quartz with magnetite during metamorphism. In comparison, as a result of meta-

morphic effects, cherts and quartz separates from Areas Two and Three are depleted in  $^{18}\text{O}$  as much as  $10.3\text{‰}$  compared to the maximum value found in sample 4-27/8-74 of Area One.

To interpret the data in Table 1, we have made four assumptions concerning the early Archaean ocean. First, the hydrosphere and atmosphere derived from volcanic emissions during the initial degassing of the earth as suggested by Fanale<sup>7</sup>. The water outgassed during this process should have a maximum  $\delta$ -value of approximately  $+6\text{‰}$ , as a result of high temperature equilibrium with igneous material. Second, oceanic water was cycled through parts of the oceanic crust allowing for isotopic exchange reactions with igneous material at high temperatures. Third, the  $\delta$ -values and environmental conditions of the early Archaean ocean are reflected in the  $\delta$ -value of the precipitated sediment, and are preserved in the rock units having the highest reported  $\delta^{18}\text{O}$  values. Finally, the mass of the Archaean ocean and the surface area of the earth, including the hydrosphere, were comparable to present day values.

Although the Isua cherts could have been precipitated under a number of different sets of conditions and still produce the same  $\delta$ -values, some realistic constraints may be placed on the palaeotemperature of the early Archaean ocean. A minimum temperature of approximately  $0^\circ\text{C}$  can be established for the Archaean ocean. The approximate lower limit is  $0^\circ\text{C}$ , since water must remain in a liquid state for precipitation of sediments. Different maximum temperatures can be achieved by assuming different initial  $\delta$ -values for the ocean. An upper temperature limit may be determined from the chert–water fractionation equation<sup>8</sup>

$$\Delta_{\text{CW}} = \frac{3.09 \times 10^6}{T^2} - 3.29$$

In the following, two different initial oceanic ratios are considered.

First, the Archaean ocean is assumed to have a  $\delta$ -value of

**Table 1** Oxygen isotope composition of chert and quartz from banded iron formations in the Isua Supracrustal belt

Sample	$\delta^{18}\text{O}_{\text{SMOW}}$	Remarks
Area One: main iron formation and associated cherts at Isua		
1-27/8-74	$+19.6 \pm 0.01$ (2)	Laminated metamorphosed chert
2-27/8-74	$+16.1 \pm 0.08$ (3)	Laminated metamorphosed chert
3-27/8-74	$+17.7 \pm 0.04$ (2)	Metamorphosed chert
4-27/8-74	$+20.3 \pm 0.13$ (2)	Metamorphosed chert
4.1-27/8-74	$+20.2 \pm 0.11$ (2)	Metamorphosed chert
4.5-27/8-74	$+19.8 \pm 0.09$ (2)	Metamorphosed chert
5-27/8-74	$+19.1 \pm 0.03$ (2)	Metamorphosed chert
7-27/8-74	$+12.6 \pm 0.02$ (2)	Quartz from banded iron formation
8-27/8-74	$+13.1$ (1)	Quartz from banded iron formation
9-27/8-74	$+15.9 \pm 0.04$ (2)	Quartz from banded iron formation
EP-11-74	$+19.1 \pm 0.03$ (2)	Metamorphosed chert
Area Two: peninsula in Lake Imarsuaq at southern tip of south-eastern limb		
EP-17-74	$+13.8 \pm 0.04$ (2)	Quartz from banded iron formation
EP-19-74	$+13.9$ (1)	Quartz from banded iron formation
EP-21-74	$+13.8 \pm 0.02$ (2)	Metamorphosed chert
Area Three: chert sequence from the north-western limb		
EP-34-74-2	$+13.5 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-3	$+13.2 \pm 0.04$ (2)	Laminated metamorphosed chert
EP-34-74-4	$+13.5 \pm 0.02$ (2)	Metamorphosed chert
EP-34-74-5	$+13.6 \pm 0.05$ (2)	Metamorphosed chert
EP-34-74-6	$+12.8 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-7	$+10.0 \pm 0.15$ (3)	Metamorphosed chert
EP-34-74-8	$+11.7 \pm 0.09$ (3)	Metamorphosed chert
EP-34-74-11	$+12.0 \pm 0.10$ (2)	Metamorphosed chert

The numbers in parentheses are the number of determinations per sample.

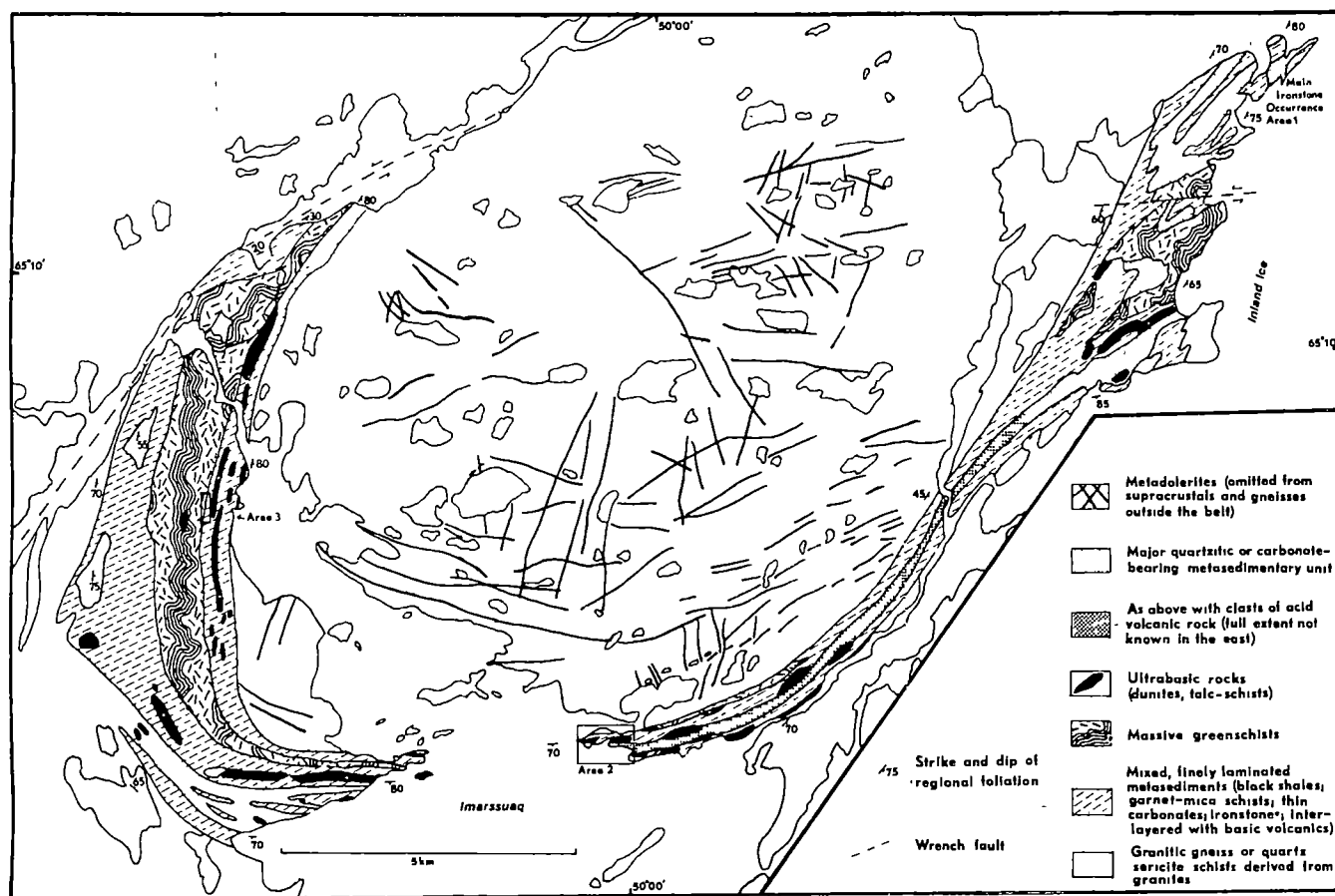


Fig. 1 Generalised geological map of the Isua supracrustal belt, West Greenland. Modified after Bridgwater and Fyfe<sup>6</sup>.

+6‰, the maximum for volcanic water in equilibrium with basalt at high temperatures. Isotopic fractionation between the Archaean ocean and the Isua chert with the maximum  $\delta^{18}\text{O}$  value (+20.3‰) is 14.3‰. This value is a minimum fractionation, and therefore, the corresponding temperature of 146 °C from the fractionation equation is an absolute maximum for the Archaean ocean. At this temperature, the partial pressure of  $\text{H}_2\text{O}$  in the atmosphere would be 4.65 atm; and 1.62% of the water in the present ocean would be required to produce this pressure.

In the second case, the  $\delta$ -value of the ocean is assumed to be 0‰, a value which has remained invariant with time. In these conditions, the ocean temperature would be ~ 89 °C and the partial pressure of water in the atmosphere equivalent to about 0.25% of the mass of the present ocean. Neither case 1 nor case 2 places any unreasonable mass deficiencies on the early Archaean ocean.

Another way (case 3) of achieving the observed  $\delta$ -values is for the Archaean ocean to have had the same temperature as now but with a  $\delta$ -value of -14‰. This assumes that the oceanic

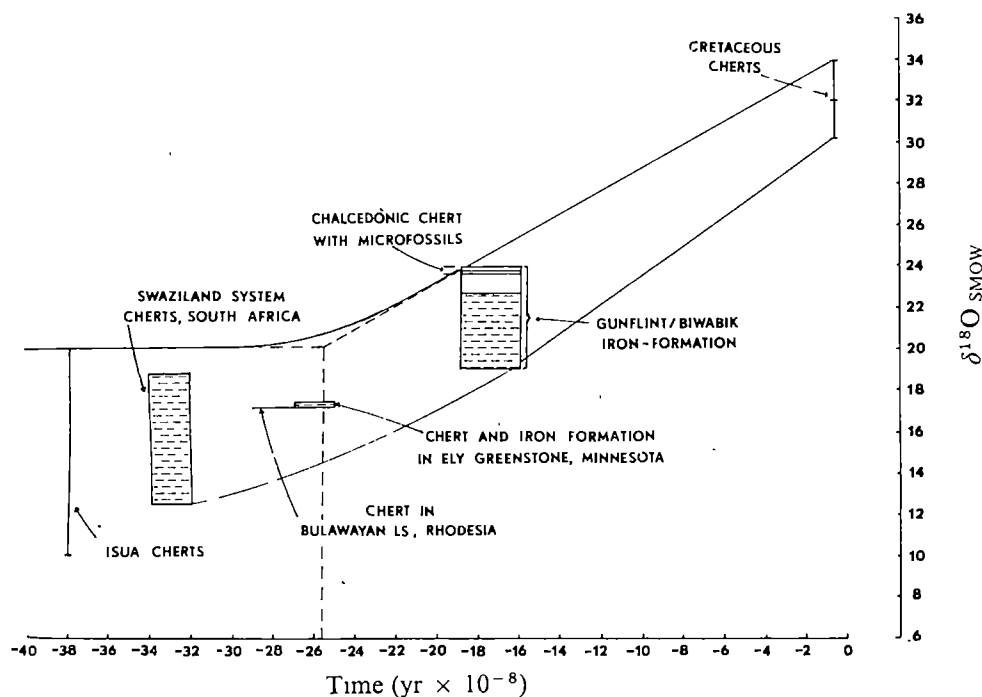


Fig. 2  $\delta^{18}\text{O}$  variations in chert-bearing units as a function of age. Cretaceous cherts, Degens and Epstein (1962); Precambrian units except Isua cherts, ref. 9; Isua cherts, this study

isotopic composition through time has paralleled the maximum values of the chert data of Perry and Tan<sup>9</sup>, but has been displaced by  $-34\%$ .

Irrespective of the initial ocean  $\delta$ -value, processes affecting the oceanic system have occurred, allowing the establishment of the present day value of  $0\%$ . If the early ocean had a  $\delta$ -value of  $+6\%$  as assumed in case 1, the most important mechanism lowering the oceanic  $\delta$ -value would be large scale, low temperature sedimentary processes. Low temperature sedimentation would allow for preferential fractionation of  $^{18}\text{O}$  to the sediment, which in turn would deplete the oceans. In a hot or warm Archaean ocean (case 1 and 2) the importance of sedimentary processes in controlling  $\delta^{18}\text{O}$  of the ocean would be enhanced relative to other processes. To maintain the ocean at  $0\%$  since the early Archaean as in case 2, long term buffering of the oceans by basic rocks would be necessary<sup>10</sup>. The only process that would account for a rise in the  $\delta$ -value of the ocean for  $-14$  to  $0\%$  would be the active interaction of ocean water and igneous rock reservoirs at high temperatures through some type of cycling process<sup>11</sup>.

Figure 2 is a plot of age against  $\delta^{18}\text{O}$  of the Isua cherts and younger metamorphosed chert units showing an apparent decrease in  $^{18}\text{O}$  with increasing age to approximately 2,600 Myr. There is a constancy in the apparent maximum  $\delta$ -value of the Archaean chert bearing units. The change in slope at  $\sim 2,600$  Myr may reflect a variation in oceanic temperature coupled with changes in the mechanism which controlled the  $\delta^{18}\text{O}$ . These changes may have arisen from variations in the interactions between the oceans and the upper mantle. Evidence has been presented by Veizer<sup>12</sup> from  $^{87}\text{Sr}$  data implying a decrease in the amount of water cycled through crustal igneous reservoirs and/or increased sedimentation. The time at which the change of slope occurs is sensitive to small variations in the maximum value of  $\delta^{18}\text{O}$ . A further study of the Isua area is under way to confirm the range of  $\delta$ -values of the Isua cherts thus allowing for further and more thorough interpretation.

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## Multiple sources of pumice in the Aegean

QUATERNARY pumice has been produced in at least ten volcanic eruptions in the southern Aegean, from volcanoes on the islands of Melos, Santorini, and various of the Dodecanese. This multiplicity of sources has not been generally recognised before, but it should be taken into account in submarine core sampling.

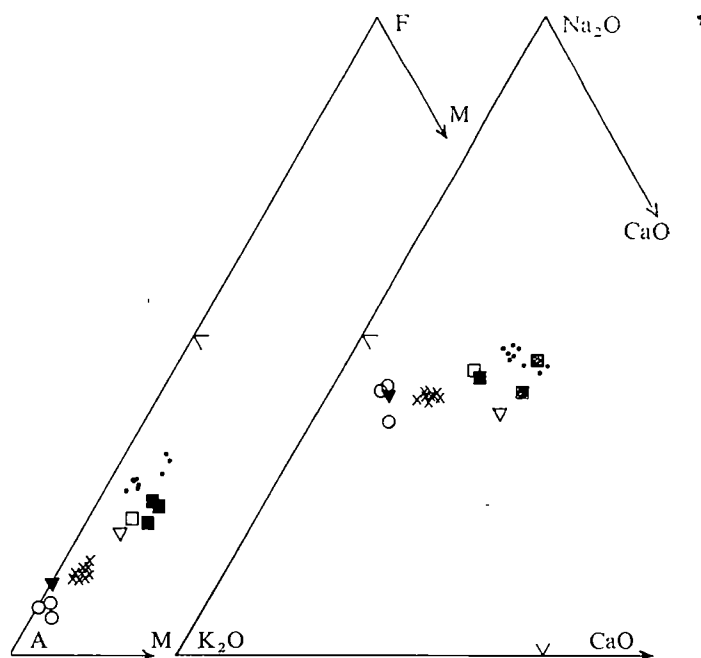


Fig. 1 Plots of chemical analyses of Quaternary pumice from various volcanoes in the Aegean. A,  $\text{Na}_2\text{O} + \text{K}_2\text{O}$ ; F, total iron as  $\text{FeO}$ ; M,  $\text{MgO}$ . •, 1470 BC Santorini, □, pumice 'G', Nisyros, ■, pumice 'A', Nisyros; ▽, pumice 'C', Yali; ▼, pumice 'D', Yali, ×, plateau pumice, Kos, Kalymnos, Pserimos and Telos; ○, Kefalos pumice, Kos.

The pumice on Melos occurs in a large ring in the south of the island. The ring, which is built up of repeated units of pyroclastic surge deposits, shows only slight erosion and therefore probably dates from the late Quaternary.

On Santorini, seven layers of pumice occur in the caldera walls at Kap Alai<sup>1</sup>. The eruptions which produced the two largest deposits, consisting of air-fall pumice and ashes, and pyroclastic flows, have been dated at 18000 BC and 1470 BC<sup>2</sup>. The older pumice deposit is locally  $>50$  m thick. Isopach maps<sup>3</sup> of the products of the younger eruption suggest that the 0.01  $T_{\text{max}}$  isopach, that is the 5.5 cm isopach, must cover several thousand  $\text{km}^2$ , and the eruption was thus of plinian type<sup>4</sup>. The isopachs are extended along a dispersal axis south-east of Santorini, and ash from this eruption has been identified from deep-sea cores from this area<sup>5</sup>.

In the Dodecanese, the products of eight pumice eruptions from three island volcanoes can be distinguished. Several pyroclastic deposits occur on Kos, and the least altered of these, here called the Kefalos deposit, occurs around the rhyolite dome of Mt Xenia. The deposit consists of repeated thin pyroclastic surge and non-welded ignimbrite flow units and covers  $>9 \text{ km}^2$ . It is overlain by the products of a much larger eruption, including pyroclastic surge deposits, ignimbrites, and associated mud-flows. This plateau deposit covers the central and western parts of Kos and is also found on the islands of Kalymnos, Pserimos and Telos, respectively 18 km north, 8 km north, and 50 km south of Kos<sup>6</sup>. On these islands the plateau deposit is confined to valleys and bays facing Kos. The products of this eruption must cover  $>2,500 \text{ km}^2$ .

On Yali, 2 km south of Kos, there are two pumice fall deposits. The older one, 'D', is  $>180$  m thick, built up of reversely-graded units dipping steeply north-east, and is possibly the remnant of a pumice ring. This is covered by a second fall deposit, 'C', which is 3 m thick. Between these two, an horizon of marine shells occurs at 170 m above sea level, showing that considerable vertical movements have taken place.

**Table 1** Percentage weights of phenocryst minerals in the Aegean pumices and refractive indices (r.i.) of glasses

	Phenocrysts (weight %)	Quartz (weight %)	Feldspar (weight %)	r.i.	Biotite (weight %)	Magnetite (weight %)	O'px. (weight %)	C'px (weight %)	Refractive index of glass
Santorini (1470 BC)	10		82	1.553			16	2	1.509
Nisyros (pumice 'G')	10		80	1.553		2	17	1	1.492
Nisyros (pumice 'A')	24		83	1.548		3	13	1	1.497
Yali (pumice 'C')	2	✓	✓	1.546					1.492
Yali (pumice 'D')	1	✓					✓		1.497
Plateau pumice, Kos	26	71	10	1.540	18	1			1.492
Kefalos pumice, Kos	8	98	2	1.540					1.501
Melos	4	92	5	1.548	2	1			1.492

✓, Present in small quantities; not measured.

Pumice from four eruptions has been preserved on Nisyros island, ~2 km south of Yali. The oldest is a pumice fall deposit exposed near the base of a cliff section within the caldera. The second fall deposit, 'G', which is rich in lithic fragments including metamorphosed limestones and plutonic rocks, is preserved mainly in the valleys on the south-west side of the island. The youngest deposit, 'A', is a pumice fall more than 20 m thick on the north-east side of the island. Pyroclastic flows which are exposed above 'G' along the north-east coastal section, seem to be the products of a fourth eruption. Fall deposits 'A' and 'G' are of plinian type.

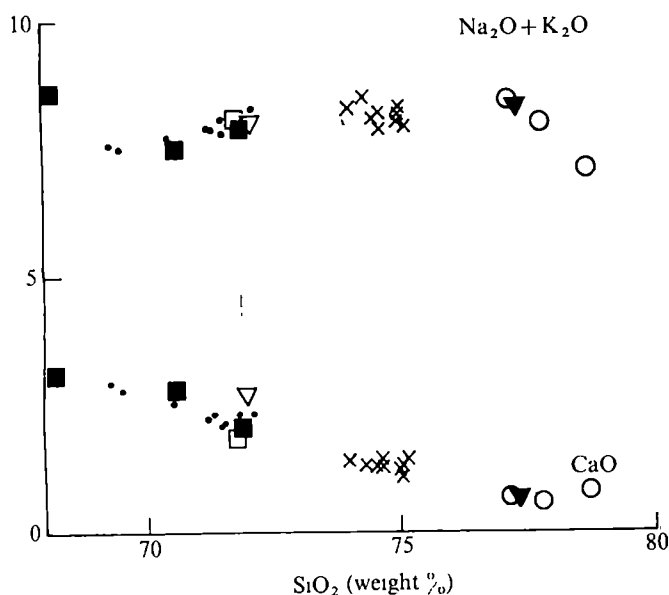
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**Fig. 2** Plots of CaO and Na<sub>2</sub>O+K<sub>2</sub>O against weight % SiO<sub>2</sub> for the same analysed samples as in Fig. 1; analyses recalculated to 100% water-free. The analyses cover the range dacite–rhyodacite–rhyolite–leucorhyolite.

The steep slopes and small size of Yali and Nisyros make it difficult to construct isopach maps of the pumice deposits. From the considerable thickness of deposits 'D' and 'A' it seems probable, however, that ashes from these islands, as well as from Santorini and Kos, should be widely found in cores from the south-east Aegean.

Major element X-ray fluorescence analyses have been made on pumice from the main eruptions to characterise these deposits. The plots of these analyses (Figs 1 and 2) illustrate the principle chemical differences. The refractive index of the glass, and the percentage and type of phenocryst minerals within the pumices have also been determined (Table 1) as an aid to the identification of the pumice from the various eruptions.

## Ocean rise-like basalts within the Galapagos Archipelago

THE Galapagos Archipelago, Iceland, and other oceanic islands, have been interpreted as surface manifestations of mantle plumes<sup>1–3</sup>. A key element of this model of island development centres on the petrological and geochemical contrasts between suites of lavas erupted from the proposed mantle plumes and at oceanic rises<sup>1–3</sup>. But the recognition of basalts of ocean rise composition on Iceland has prompted modification of models of petrological processes in mantle plumes<sup>4,5</sup>. We know of only one other reported occurrence of ocean rise-like basalts erupted on islands of proposed plume origin: flows in the Pololu Series on the Island of Hawaii<sup>6</sup>.

Here we report a newly discovered occurrence of ocean rise-type basalts erupted on Santiago Island, Galapagos. The presence of these distinctive rocks is an important factor in interpreting the possible roles of mantle plume activity or other tectonic forces in the development of the Galapagos Archipelago.

Ocean rise basalts are typified by consistently low abundances of incompatible elements (such as, K<sub>2</sub>O, TiO<sub>2</sub>, and P<sub>2</sub>O<sub>5</sub>), (refs 3, 7 and 8). Most ocean rise basalts are devoid of clinopyroxene phenocrysts, but commonly contain olivine and/or plagioclase phenocrysts<sup>3,8</sup>. Some pyroxene phenocrysts have, however, been observed in samples dredged from the rift valley of the Galapagos Rise<sup>2,9</sup>. The compositional variation among ocean rise basalts, though quite small, has been attributed to fractional crystallisation<sup>7,9</sup>, to varying degrees and depths of partial melting<sup>3</sup>, and to differences in the compositions of parental material<sup>10</sup>.

The same mechanisms have also been proposed as the source of more pronounced differences between ocean rise basalts and plume-derived lavas<sup>3,11,12</sup>. The consistently low abundances of incompatible elements in ocean rise basalts contrast with the much greater compositional and petrographic variation and generally higher abundances of these elements in lavas erupted on oceanic islands of proposed plume origin.



Table 1 Major element analyses of selected basalts from the Galapagos Islands and the Galapagos Rise

	1*	2	3	4	5	6	7	8
	JL-74	JL-122	JH-86	JH-115	Ave-K	Ave-A	Ave-T	Ave-D7
SiO <sub>2</sub>	46.80	47.06	46.46	46.87	46.75	46.81	48.36	49.60
TiO <sub>2</sub>	1.84	1.37	1.26	1.43	1.43	2.49	2.65	1.02
Al <sub>2</sub> O <sub>3</sub>	16.22	16.71	16.00	16.42	16.46	15.77	14.53	15.97
Fe <sub>2</sub> O <sub>3</sub>	1.84	2.65	2.95	3.81	2.58	3.62	3.32	
FeO	8.77	7.62	7.37	7.01	7.82	8.58	8.79	8.76
MnO	0.19	0.18	0.18	0.18	0.18	0.20	0.18	0.15
MgO	8.18	9.50	10.79	9.52	9.58	7.74	6.63	7.83
CaO	12.47	12.52	11.97	11.74	12.19	10.28	11.45	12.94
Na <sub>2</sub> O	2.36	2.45	2.28	2.38	2.42	3.12	2.69	2.43
K <sub>2</sub> O	0.07	0.03	0.06	0.15	0.07	0.44	0.36	0.08
P <sub>2</sub> O <sub>5</sub>	0.11	0.07	0.10	0.12	0.10	0.32	0.25	0.07
H <sub>2</sub> O <sup>-</sup>							0.17	
H <sub>2</sub> O <sup>+</sup>	0.35†		0.27†	0.06†	0.17†	0.22†	0.61	
Total	99.20	100.16	99.71	99.68	99.75	99.59	99.99	98.95
FeO*/MgO	1.27	1.05	0.93	1.10	1.06	1.53	1.78	1.12

\*1-4, Representative low-K<sub>2</sub>O lavas from Santiago Island; 5, average analysis of 8 low-K<sub>2</sub>O lavas from Santiago Island; 6, average analysis of 17 alkalic lavas from Santiago Island; 7, average Galapagos tholeiite, from ref. 13; 8, average analysis of 4 basalts dredged from the Costa Rica Rift Zone, Galapagos Rise, based on data from ref. 9.

†Total H<sub>2</sub>O loss on ignition at 500 °C.

Petrographically and compositionally, the low-K<sub>2</sub>O lavas erupted on Santiago are essentially identical to olivine tholeiites sampled from the East Pacific Rise, the Galapagos Rift and other active spreading centres (Table 1 and Fig. 1). The Santiago lavas contain up to 10% phenocrysts of olivine and plagioclase in a matrix of plagioclase laths, clinopyroxene, olivine and opaques. No pyroxene phenocrysts were observed in the eight samples we examined.

The ocean rise-type lavas from Santiago are characterised by much lower abundances of the incompatible elements and higher MgO, CaO and Al<sub>2</sub>O<sub>3</sub> than either average Galapagos Island tholeiite<sup>13</sup> or the more alkalic lavas also found on Santiago (Table 1). Variation diagrams of selected depleted elements against the ratio FeO\*/MgO (ref. 14) show that the low-K<sub>2</sub>O lavas are clearly disjunct from suites of other Galapagos Island rocks (Fig. 1).

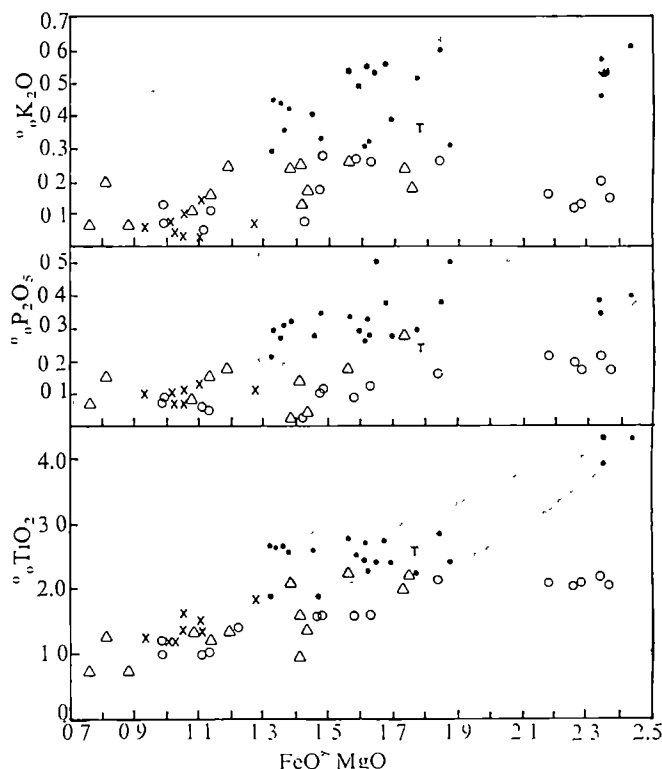
Several petrological features of the ocean rise-type basalts on Santiago indicate that they are the product of a distinct process taking place beneath the central area of the Galapagos platform. The low-K<sub>2</sub>O lavas are younger than or coeval with the alkalic rocks which form the bulk of the exposed portion of Santiago. This is the reverse of the typical tholeiitic-alkalic lava sequential history of Hawaiian volcanoes<sup>15</sup>. Furthermore, it is unlikely that the ocean rise-type basalts are genetically related to the older or contemporaneously erupted alkalic lavas by any sort of crystal fractionation process<sup>3,10,16</sup>.

Structural features associated with the ocean rise-type lavas suggest that these petrological features are related to a relatively young phase of tectonic activity in the central and eastern portion of the Galapagos Archipelago. The low-K<sub>2</sub>O lavas on Santiago were erupted from fissures which are part of an easterly trending structural pattern of aligned vents, normal faults and fissures, and uplifted submarine lavas observed on seven islands in the central and eastern portion of the archipelago (Fig. 2)<sup>17</sup>. This structural pattern postdates the construction of the principal volcanoes forming Santiago, Santa Cruz and San Cristobal. On islands where the structural features can be dated, their ages are less than about 0.5 Myr. The ocean rise-type lavas on Santiago were erupted over the past several hundred thousand years and as recently as 1906.

The fault patterns and uplifted lavas record uplift and north-south distention in a zone extending eastward from the centre of the archipelago<sup>17</sup>. This zone straddles an east-west trending negative gravity anomaly<sup>18</sup>. Although scanty data preclude an unambiguous explanation of the

gravity low, it has been interpreted as the result of an elongate east-west curtain of mantle upwelling beneath the archipelago<sup>18,19</sup>. Such a zone of upwelling would parallel the zone of active seafloor spreading at the Galapagos Rift 150 km to the north (Fig. 2)<sup>20</sup>. The close spatial and temporal relationship among the subaerially erupted, ocean rise-type basalts on Santiago, the zone of uplift and north-south distention, and the negative gravity anomaly, suggest there has been a relatively recent (0.5 Myr BP to present) period of mantle upwelling beneath the central and eastern portion of the Galapagos Archipelago. Perhaps these features signal incipient rifting of the Galapagos Platform

Fig. 1 Abundances of K<sub>2</sub>O, P<sub>2</sub>O<sub>5</sub> and TiO<sub>2</sub> plotted against the ratio FeO\*/MgO for basalts from the East Pacific Rise<sup>2,8</sup>. Δ, Galapagos Ridge rift valley<sup>9</sup>; ○, Galapagos Island average tholeiite<sup>13</sup> (T), Santiago Island low-K<sub>2</sub>O lavas (×), and alkalic lavas (● and shaded area), (H.W.B., unpublished).



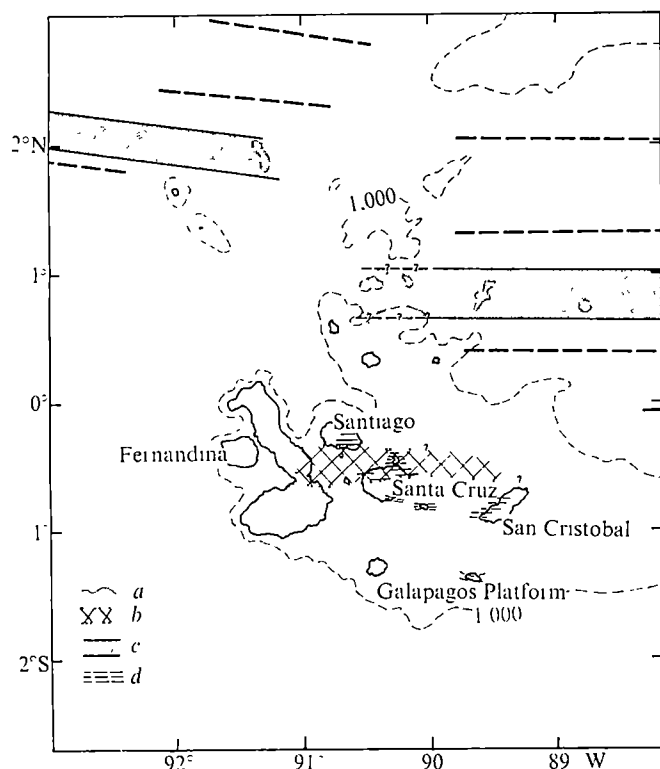


Fig. 2 Galapagos Archipelago. a, 1,000 fathom contour; b, axis of negative gravity anomaly<sup>18</sup>; c, Galapagos Rift<sup>20</sup>; d, aligned vents, normal faults, and fissures<sup>17</sup>.

by ocean rise-type processes in a fashion similar to the splitting of the ancestral Carnegie Ridge (see ref. 21).

The recent petrological-tectonic history of the central Galapagos could also be interpreted using the currently popular model of mantle plumes. Using a mantle plume hypothesis analogous to the model proposed for Iceland and the Reykjanes Ridge by Sigvaldason *et al.*<sup>4</sup>, the ocean rise-type lavas could be derived from source material progressively depleted during lateral plume flow eastwards from a hot spot at present centred beneath Fernandina Volcano. Other models would derive both low-K<sub>2</sub>O lavas and typical oceanic island basalts from different regions within the column of the upwelling plume<sup>5</sup>.

We feel, however, that 'hot spot' and mantle plume models of island development contribute little to our understanding of the history of the Galapagos Archipelago. Clearly, the available geological and geochemical data are insufficient to devise a realistic, unambiguous petrological-tectonic model for the Galapagos region. As in the case of Iceland<sup>22</sup> the Galapagos Islands seem to have had a complex history influenced by several distinct but possibly superimposed petrological and tectonic regimes.

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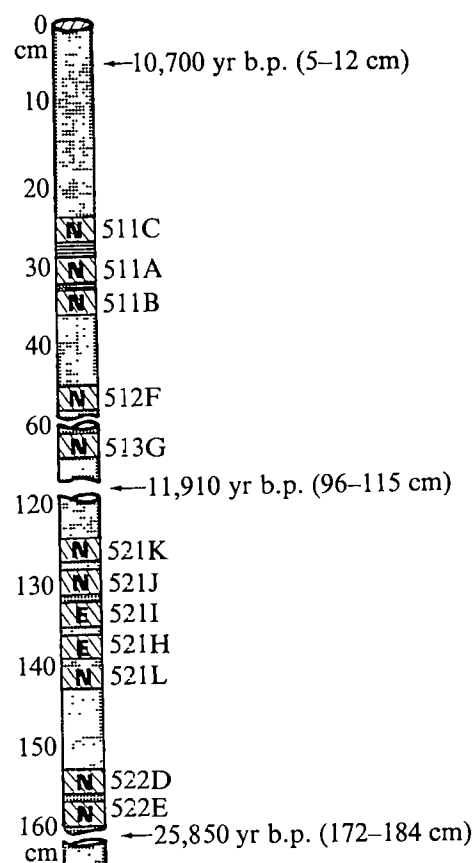
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## Geomagnetic excursion from Imuruk Lake, Alaska

UNGLACIATED Arctic lake sediments probably offer the best opportunity for correct stratigraphic correlation and radiocarbon dating of brief geomagnetic events.

We possess a series of lake sediment cores from Imuruk Lake, Seward Peninsula, Alaska, which represent a time interval well beyond the range of radiocarbon dating<sup>1</sup>. Pollen analysis of Arctic Alaskan lake sediments can be used to identify glacial and non-glacial episodes with considerable precision<sup>2</sup> and the pollen record from one Imuruk Lake core suggests that at least one, and perhaps two, complete glacial cycles are included<sup>3</sup>. Our suite of cores thus offers an opportunity to study the history of palaeo-magnetic events over a time interval of perhaps 200,000 yr. We report here the discovery of the Imuruk geomagnetic excursion some 18,000 yr b.p.

Fig. 1 Sampling intervals and geomagnetic excursion interval in Imuruk Lake core IV. N, Normal polarity samples; E, geomagnetic excursion samples. Radiocarbon dates as in Table 1.



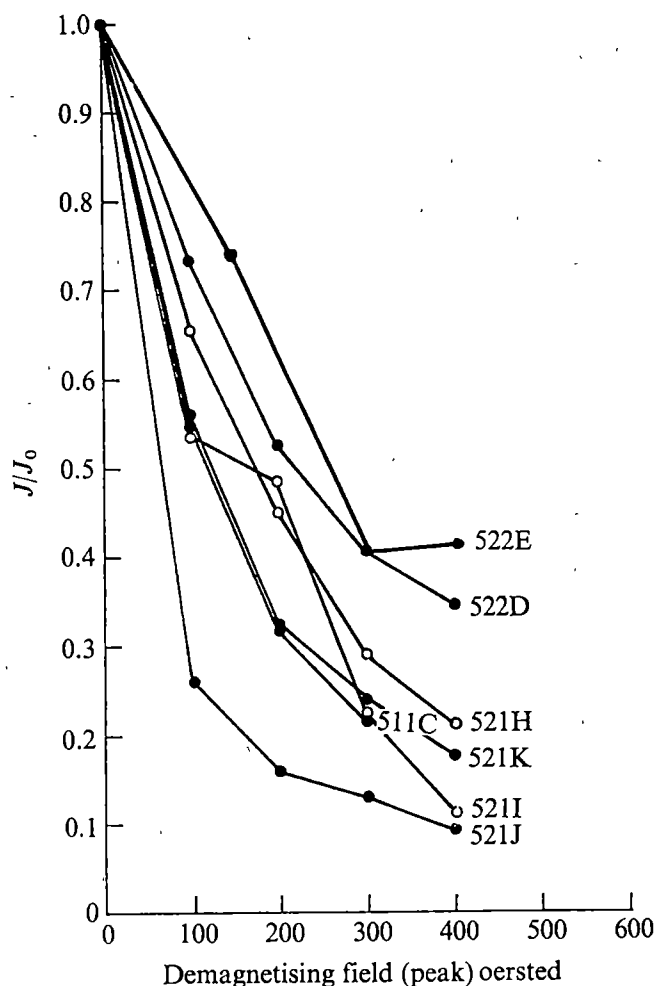
**Table 1** Radiocarbon dates on Imuruk Lake core IV

5-12 cm (I-3631)	10,700 ± 150 yr b.p.
96-115 cm (I-3632)	11,910 ± 180 yr b.p.
172-184 cm (I-3662)	25,850 ± 2,400 yr b.p.

The discovery was made with Imuruk Lake Core IV (coordinates 65°37' N, 163°8' W) collected in August, 1960 (ref. 1). The core was stored in the Dural sample tubes with which it was taken until 1967, when it was extruded and examined using radiocarbon dating and pollen analysis<sup>3</sup>. Since then, the core has been wrapped in Saran film and stored at 4 °C. Core IV is short (2.5 m), but it includes pollen zones, J, K, and L, representing glacial and early postglacial time<sup>1-3</sup>. Three radiocarbon dates (Table 1) show that the core spans the interval from before 25,000 yr b.p. to about 10,000 yr b.p. This interval spans the estimated ages of a number of geomagnetic excursions, noticeable among them that from Lake Biwa at 18,000 yr b.p. (ref. 4). We found the dating of the Biwa Event particularly interesting because it is synchronous with the last glacial maximum. Accordingly, we chose for our reconnaissance samples the interval of Imuruk core IV in which the sediments of 18,000 yr b.p. must lie.

We removed twelve core samples from the upper 160 cm of Imuruk Core IV (Fig. 1). The cores were measured using a Schonstedt SSM-1A spinner magnetometer. Following the measurement of the natural remanent magnetisation (NRM), the cores were subjected to successive stages of alternating field demagnetisation (a.f.d.) using a Schonstedt GSD-1 demagnetising unit. The total moments of the NRM averaged  $5.0 \times 10^{-3}$  gauss cm<sup>3</sup>, with individual core volumes of 10 cm<sup>3</sup>. Ten cores had NRM inclinations averaging 45° and may be considered of normal polarity. Two adjacent cores at 132-135 cm and 136-139 cm (Fig. 1) had NRM inclinations averaging 13°. All of the NRM moments had a similar magnitude suggesting that the NRMs of the two cores with low inclination record a sudden excursion of the geomagnetic field.

We tested the stability of NRM of the samples by demagnetising them in an alternating field to a peak of 400 oersted, in stages of 100 oersted. After a.f.d. to 400 oersted all of the samples showed the same rate of decrease of residual moment to about 25% of their initial values, which suggests that their magnetic mineralogy is uniform (Fig. 2, Table 2). After demagnetisation, the residual inclinations of the samples with normal polarity averaged 48°, a few degrees more than their average NRM inclination. The two samples with low NRM inclinations, however, had residual magnetisations nearly horizontal, 13° less than their average NRM inclination. This indicates that the stable NRM moments in the samples are free of



**Fig. 2** Alternating field demagnetisation results. ●, Samples with normal (positive) magnetic inclinations; ○, samples with nearly horizontal (excursion) inclinations.  $J/J_0$  is the ratio of NRM to the residual moment after a.f.d.

a consistent bedding plane error, and that there was a change of geomagnetic inclination of approximately 50°. The magnitude of this change is close to the 55° change recorded by Yaskawa *et al.*<sup>4</sup> from the Lake Biwa (35°13' N, 136°01' E) results. This change was sufficiently abrupt to have occurred between adjacent samples (Fig. 1), suggesting that the decrease in inclination was very rapid, a circumstance which may be tested by investigating the rate at which the Imuruk lake sediments accumulated. It is most unlikely that any of the core samples acquired their NRM long after initial deposition. (Verosub<sup>5</sup> reports

**Table 2** NRM (a) and 400-oersted (b) residual magnetisation results\* for seven samples of Imuruk Lake core IV

	Declination (degrees)	Inclination (degrees)	Specimen	Total moment (e.m.u.)
a	206.68	53.22	I4 511C	$9.02 \times 10^{-6}$
	204.09	47.53	I4 522D	$6.17 \times 10^{-6}$
	204.30	50.73	I4 522E	$3.36 \times 10^{-6}$
	171.50	9.12	I4 521I	$1.39 \times 10^{-6}$
	188.61	17.28	I4 521H	$8.92 \times 10^{-6}$
	178.07	45.09	I4 521J	$5.48 \times 10^{-6}$
	199.17	42.11	I4 521K	$9.13 \times 10^{-6}$
b	131.55	70.45	I4 511C	$2.02 \times 10^{-6}$
	214.95	53.25	I4 522D	$2.12 \times 10^{-6}$
	175.67	45.21	I4 522E	$1.41 \times 10^{-6}$
	173.85	-0.16	I4 521H	$1.84 \times 10^{-6}$
	184.98	0.49	I4 521I	$1.47 \times 10^{-6}$
	169.24	35.65	I4 521J	$5.17 \times 10^{-6}$
	203.18	58.14	I4 521K	$1.60 \times 10^{-6}$

\*The results are plotted in Figure 2. The coring tube was not oriented for azimuth and the declinations are not correct in geographic coordinates

finding stable NRM in lake sediments acquired within 5 years of deposition.) The conditions in which sediment collects at Imuruk Lake<sup>1</sup> preclude the possibility of small scale folding of the deposit similar to that reported by Verosub<sup>5</sup> for a glacial lake in Massachusetts which locally reproduced the effect of a geomagnetic excursion. Furthermore, the part of the Seward Peninsula containing Imuruk Lake was free of ice during the Wisconsin glaciation<sup>9</sup>. We conclude that the samples record the orientation of the local geomagnetic field at the time of deposition, and that the low inclination of the two cores represents a real geomagnetic excursion. We have not yet identified the magnetic minerals in the core, but the data are consistent with detrital magnetite contributing about 50% of the NRM, as has been noted in other Recent lake sediments<sup>6-8</sup>.

The average inclination of the stable NRM moments ( $48^\circ$ ) in the majority of the samples is about  $27^\circ$  less than that expected at the latitude of  $65^\circ\text{N}$ , assuming a geocentric dipole source for the main geomagnetic field. We can reduce the discrepancy to  $22^\circ$  because of a  $5^\circ$  tilt of the lake bed to the south about 10,000 yr b.p.<sup>9</sup>. The inclination of the stable NRM of the majority of the Lake Biwa samples<sup>4</sup> during the equivalent time interval (11,000–24,000 yr b.p.) averages about  $48^\circ$ ,  $5^\circ$  less than expected for a geocentric dipole at  $35^\circ\text{N}$ . The present-day geomagnetic inclination in the Seward Peninsula region is  $75^\circ$  (ref. 10), and there is no evidence of a localised anomalous vertical intensity of  $-11,000\text{ }\gamma$  near Imuruk Lake, which would be required systematically to diminish by  $20^\circ$  the local geomagnetic inclination during a normal polarity epoch. The low value we observe for the stable NRM inclination may be the result of our small sample, or there may have been a low geomagnetic inclination in the North Pacific region during the Wisconsin Glaciation. The presence of a geomagnetic excursion involving a change in inclination of  $50^\circ$  is, however, clear.

If we assume that the accumulation of sediment between the radiocarbon dates bounding the event (Table 1) is linear, the sedimentation rate during the period of the event is about 1 cm every 193 yr. We can identify the approximate position of the polarity event in other cores using pollen stratigraphy, because the event lies nearly in the middle of pollen sub-zone  $J_3$  (refs 1–3). In Imuruk Core I, taken about one mile from Core IV, the middle of  $J_3$  lies at a depth of 90 cm. This lies between the radiocarbon age of  $13,250 \pm 700$  yr b.p. at 50–60 cm, and more than 34,500 yr b.p. at 110–120 cm (ref. 7). Assuming a linear sedimentation rate between these ages, the minimum rate would be 7 cm every 300 yr. In Imuruk Core II, taken a mile from cores I and IV, the middle of sub-zone  $J_3$  lies at a depth of 60 cm and is bounded by radiocarbon ages of  $12,160 \pm 300$  yr b.p. at 5–10 cm, and more than 28,700 yr b.p. at 100–114 cm. Assuming a linear sedimentation rate gives a minimum rate of about 1 cm every 200 yr.

The interval of time spanning these pairs of dates includes both intervals of maximal glacial advance and of glacial retreat, so that some fluctuations in the rate at which sediments accumulated must be expected. The assumption of linearity in sedimentation rate cannot, therefore, be valid. Nonetheless the sediments enclosing the geomagnetic excursion in Core IV must have accumulated at a rate of 1 cm about every two or three centuries. At the onset and close of the excursion (Fig. 1) there was a change in the geomagnetic inclination of  $50^\circ$  within 1 cm of sediment (a time interval of 200–300 yr). The excursion persisted during the accumulation of 9 cm of sediment, a time interval of 1,800–2,700 yr.

Our closest estimate for the actual radiocarbon age of the excursion can be obtained from Fig. 3. Figure 3a describes the accumulation of sediments in Lake Biwa<sup>11,12</sup>, and shows an exponentially changing relationship of age

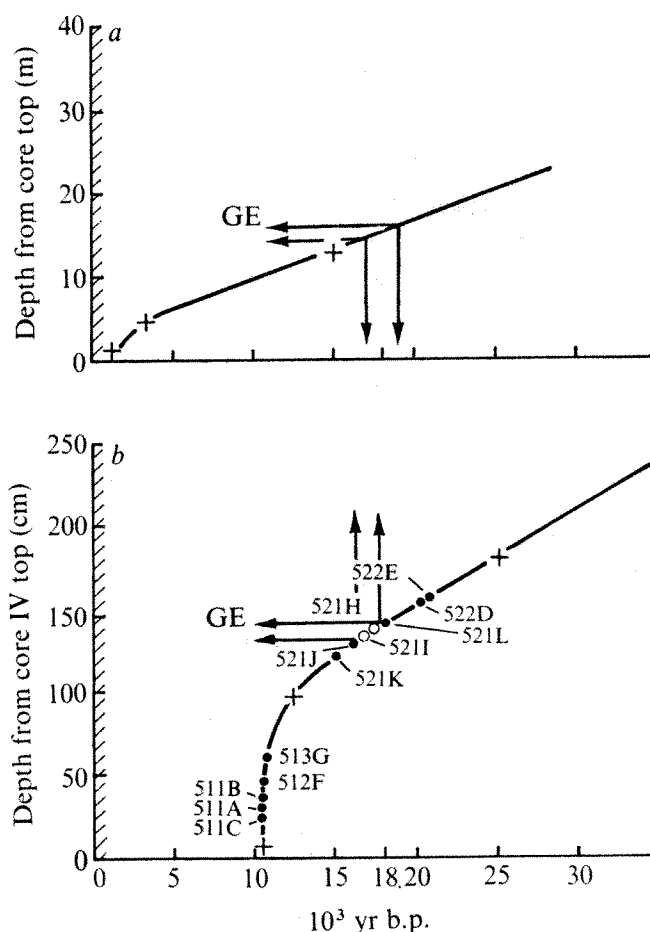


Fig. 3 Sediment accumulation and geomagnetic excursion interval at a, Lake Biwa<sup>9</sup> and b, Imuruk Lake. ●, Position of normal polarity samples; ○, geomagnetic excursion samples; crosses, radiocarbon dates; GE, geomagnetic excursion.

against depth as the sediments compact, eventually becoming linear.

Figure 3b is a similar diagram for Imuruk Lake Core IV, based on the radiocarbon dates given in Table 1. The steep age-against-depth relationship in the upper 100 cm of the core mainly reflects the truncation of the lake sediment by wave action in the shallow water of the contemporary lake<sup>9,13</sup>. The positions of the samples are indicated, and the age of the excursion is between 17,000 and 18,000 yr b.p.

It seems likely that much of the scatter in the ages of polarity events is a consequence of uncertainties in radiocarbon age determinations, or they could arise from linear extrapolation of age when the age-depth relationship is not linear. In the examples of Lakes Biwa and Imuruk, such a linear extrapolation in sediments near the top of the core would result in an underestimate of the age of the polarity event. We speculate that some of the polarity events reported for the 8,000–20,000 yr b.p. interval may be more correctly dated in the 17,000–19,000 yr b.p. interval.

It should be possible to detect the 18,000 yr b.p. geomagnetic excursion (and probably others) in equatorial sediments or igneous rocks where the normal geomagnetic inclinations are close to horizontal, even when cores are not oriented in the horizontal plane. This is because the excursion is accompanied by an apparent shift of the geomagnetic dipole axis by about  $30\text{--}35^\circ$ , and by an inclination change of  $50\text{--}55^\circ$ , which is somewhat different from the changes accompanying a geomagnetic polarity reversal. A geomagnetic reversal involves a symmetrical change of



inclination and declination, the inclination changing sign and the declination changing by  $180^\circ$ . It is not necessary to know the declination of a sample if the inclination change is approximately  $50^\circ$  at any latitude during an excursion.

P. Colbaugh undertook the pollen analyses of Core IV and R. Marino assisted with the sub-sampling of the Cores and the magnetometry. The work was supported by the NSF.

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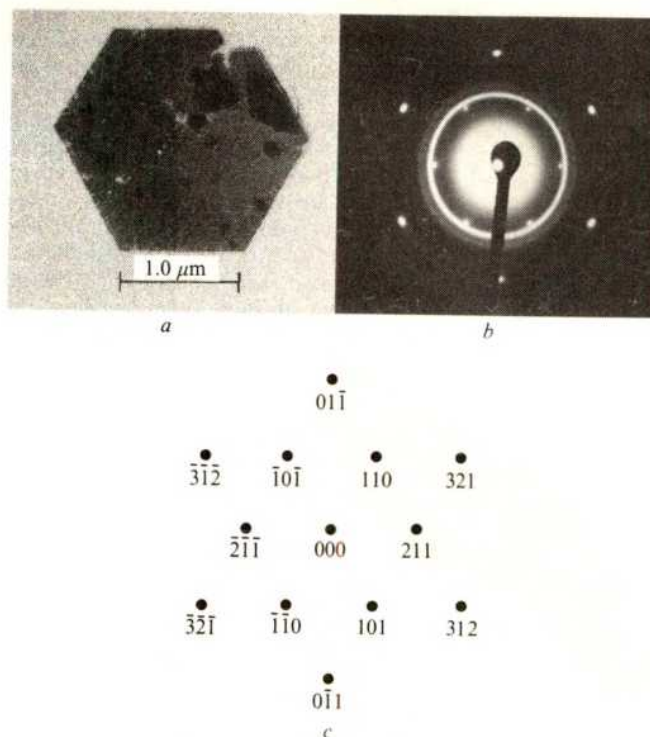
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## Crystal structure of green rust formed by corrosion of cast iron

THE principal solid corrosion products of iron in water are magnetite and hydrated forms of ferric oxide, depending on the degree of oxidation. The occurrence of intermediate products, termed green rusts, was reported by Keller<sup>1</sup> in 1948, since when the formation and decomposition of these compounds, precipitated from aqueous solution by controlled oxidation of ferrous hydroxide, have been extensively studied<sup>2–5</sup>. It has been proposed<sup>2,3</sup> that specific anions are incorporated in the crystal structure of some green rusts, although no substantial evidence has been found to confirm this. A number of green rusts have been classified crystallographically<sup>3</sup> as follows: first, rhombohedral green rusts I (GRI) formed in  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$  and  $\text{Br}^-$  solutions and (second), hexagonal green rust II (GRII) formed only in  $\text{SO}_4^{2-}$  solutions by decomposition of GRI. The interrelationships between the various products involved in the oxidation of ferrous hydroxide have been summarised by Misawa *et al.*<sup>5</sup> In this paper a preliminary account is given of the crystal structure of a rhombohedral green rust formed by corrosion of iron which, although of

**Table 1** X-ray diffraction pattern analysis of green rust on hexagonal and rhombohedral crystal systems

Line number	$d(\text{\AA})$	$(hkl)_H$	$(hkl)_R$	$I/I_0$
1	7.504	003	1 1 1	100
2	3.755	006	2 2 2	25
3	2.718	101	1 0 0	3
4	2.666	012	1 1 0	20
5	2.462	104	2 1 1	10
6	2.343	015	2 2 1	15
7	2.086	107	3 2 2	2
8	1.964	018	3 3 2	15
9	1.740	10, 10	4 3 3	5
10	1.641	01, 11	4 4 3	3
11	1.584	110	1 0 1	5
12	1.549	113	2 1 0	5
13	1.462	116	3 2 1	5

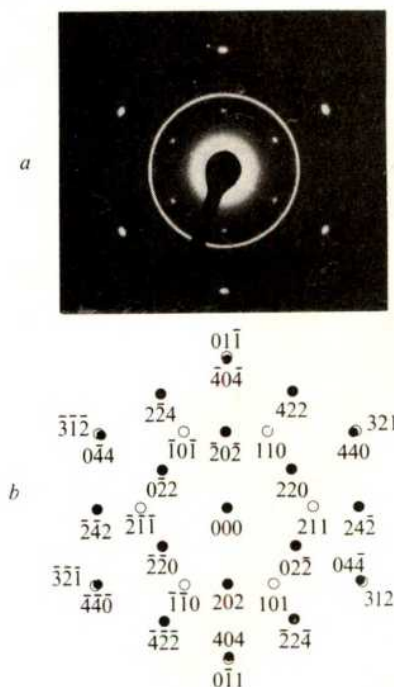


**Fig. 1** *a*, Transmission electron micrograph of green rust single crystal. *b*, Corresponding electron diffraction pattern. *c*, Indexed diagram.

similar unit cell dimensions to GRI, differs structurally from those previously reported.

Preliminary corrosion experiments were conducted on cast iron in waters containing  $\text{NaCl}$ ,  $\text{K}_2\text{SO}_4$ ,  $\text{Na}_2\text{CO}_3$ , mixtures of these salts and  $\text{NaOH}$  under various degrees of aeration. Optical microscopy showed that the corrosion products contained varying amounts of  $\text{Fe}_3\text{O}_4$ ,  $\gamma\text{-FeOOH}$  and a green compound presumed to be a green rust. X-ray powder diffraction confirmed the presence of  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-FeOOH}$ , but

**Fig. 2** *a*, Transmission electron diffraction pattern of green rust and magnetite. *b*, Indexed diagram:  $\circ$ , green rust;  $\bullet$ ,  $\text{Fe}_3\text{O}_4$ . Indices for  $\text{Fe}_3\text{O}_4$  may be deduced by reference to Fig. 1.





additional diffraction lines could not be matched with published lines for GRI or GRII. The diffraction pattern for the green compound was found to be independent of the type and combination of anion present in solution and also found to be unaffected by the level of dissolved oxygen. An oxygenated solution of  $\text{Na}_2\text{CO}_3$  ( $\text{CO}_3^{2-} = 200$  p.p.m. by weight) proved to be a convenient medium for growth of the green compound with relatively small quantities of  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-FeOOH}$  to cause interference. Thirteen unassigned lines obtained in the X-ray powder spectrum for this corrosion scale may be indexed in the rhombohedral system as shown in Table 1. Unit cell dimensions calculated from these data are  $a_R = 7.5 \pm 0.26 \text{ \AA}$  and  $\alpha = 21.52 \pm 1.15^\circ$ ; equivalent hexagonal dimensions are  $a_0 = 3.181 \pm 0.04 \text{ \AA}$  and  $c_0 = 21.82 \pm 0.8 \text{ \AA}$ . X-ray diffraction lines, ascribed by Butler and Beynon<sup>6</sup> to a chloride green rust in studies of corrosion of mild steel in boiling salt solutions, are closely related to those shown in Table 1.

Scanning electron microscopy shows that the green compound comprises clusters of flat crystals with a well-formed hexagonal

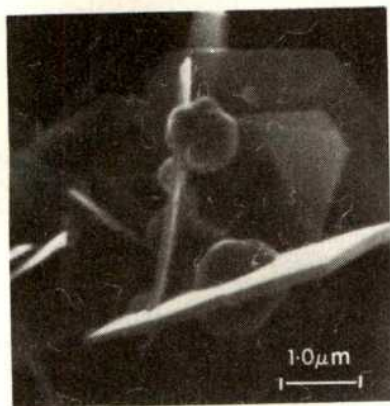


Fig. 3 Scanning electron micrograph showing magnetite growth on hexagonal green rust crystals.

habit. Single crystals were extracted from the corrosion product and prepared for transmission electron microscopy by methods to be described elsewhere. Figure 1a shows a transmission electron micrograph of a single crystal of the green compound and Fig. 1b the corresponding selected area electron diffraction pattern with a superimposed ring pattern from gold used as an internal standard. Figure 1c shows the indexed diffraction pattern. The beam direction is  $[1\bar{1}\bar{1}]_R$ ; two planes of the set  $\{211\}_R$  and four planes of the set  $\{110\}_R$  are diffracting and have similar  $d$  spacings giving the inner group of six spots; the outer group comprises two further reflections from  $\{110\}_R$  and four from  $\{321\}_R$ . The habit plane of the crystal, which is normal to  $(\bar{1}\bar{1}\bar{1})_R$  is most closely represented as  $\{5\bar{6}\bar{6}\}_R$ , and is  $5^\circ 48'$  from the hexagonal basal plane,  $(0001) \equiv (111)_R$ .

Diffraction from certain crystals of the green compound shows a second pattern superimposed on the green rust pattern (Fig. 2a). This can be indexed (Fig. 2b) as magnetite, evidently growing on the surface of the green compound. The orientation relationship between the green compound and magnetite is  $[1\bar{1}\bar{1}]_R \parallel [1\bar{1}\bar{1}]_C$  and  $(5\bar{6}\bar{6})_R \parallel (1\bar{1}\bar{1})_C$ . Morphological evidence for an epitaxial growth of magnetite on the green compound is shown in Fig. 3, in which magnetite crystallites occur on opposite faces of the hexagonal crystals.

It is concluded that the green compound formed by corrosion of iron differs structurally from green rusts I and II produced by solution precipitation. The compound is remarkably stable, and has been found coexisting with  $\text{Fe}_3\text{O}_4$  and  $\gamma\text{-FeOOH}$  in highly oxidising conditions. It is believed that the compound has a significant role in the formation of conventional scales on ferrous surfaces in aqueous environments. Work is in hand on the chemical analysis of the compound.

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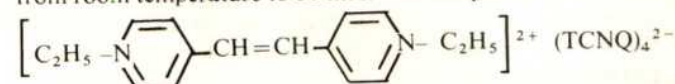
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## Organic complex radical anion salt metallic down to 30 mK

THE classical, but still recent, example of an organic metal, TTF-TCNQ, has been fully investigated and reviewed by two groups<sup>1,2</sup>. It has a room temperature electrical conductivity of  $\sim 10^3 \Omega^{-1} \text{ cm}^{-1}$  which increases on cooling to a maximum, with  $\sigma_m/\sigma_{RT} \sim 10$ -15, at  $\sim 58 \text{ K}$  (ref. 1), where it undergoes a metal-semiconductor transition. In 5 of 150 crystals,  $\sigma_m/\sigma_{RT} > 20$ -150 has been reported<sup>1</sup>. Recently<sup>3</sup> a congener HMTSF-TCNQ (hexamethylene tetraselenafulvalinium tetracyanoquinodimethanide) was reported to have  $\sigma_{RT} = 1,391$ -2,178  $\Omega^{-1} \text{ cm}^{-1}$  increasing to  $\sigma_m/\sigma_{RT} \sim 3.4$  at 75-45 K, when it decreases to  $\sigma/\sigma_{RT} = 0.3$  at 1.5 K. These organic substances showed no superconducting transition, in contrast with the inorganic polymer (SN)<sub>x</sub> which, while mostly behaving similarly<sup>4</sup>, also shows the onset of superconductivity at 0.26 K (ref. 5).

Here we report a new organic metal, the first organic complex ever to show a monotonic increase in conductivity on cooling from room temperature to 30 mK. The complex is



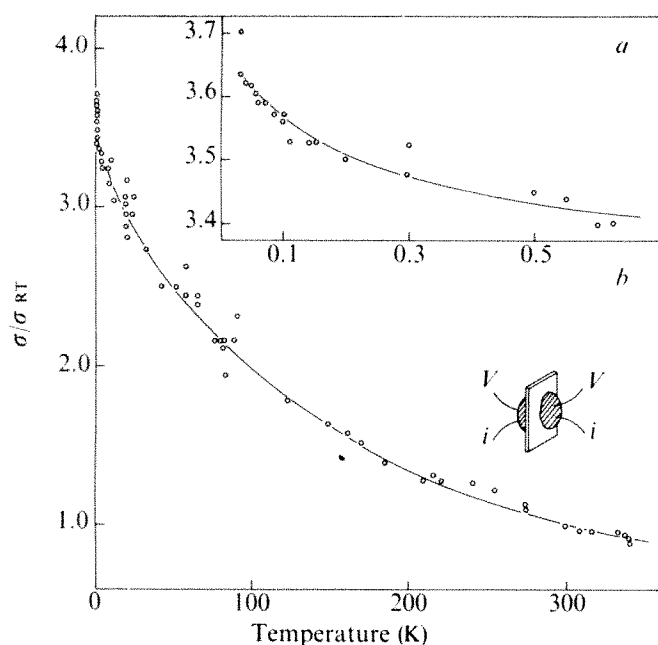
1,2-di(N-ethyl-4-pyridinium)ethylene<sup>2+</sup> (7,7,8,8-tetracyanoquinodimethane)<sub>4</sub><sup>2-</sup> which we abbreviate to (DEPE)<sup>2+</sup>(TCNQ)<sub>4</sub><sup>2-</sup>.

The complex, prepared in a similar manner to its semi-conducting congeners<sup>6</sup>, was grown as single crystals from acetonitrile, and the stoichiometry determined by micro-analysis and spectral examination in the visible region<sup>7</sup>. Semi-conducting and metallic forms of (DEPE)<sup>2+</sup>(TCNQ)<sub>4</sub><sup>2-</sup>, each with the same stoichiometry, were obtained. The conditions of preparation still remain to be determined completely.

Current and voltage probes were attached to opposite faces of the crystals, as shown in Fig. 1, with Acheson high conductivity silver paint as electrodes. A constant current was applied and the voltage drop measured by using a Keithley 640 electrometer. The electrode resistance was insignificantly small compared with the sample resistance throughout the temperature range studied.

In this communication we report the preliminary d.c. conductivity data on nine metallic single crystals (mean dimensions  $4 \times 2 \times 0.5 \text{ mm}$ ) isolated from two batches. The conductivities at 300 K of (DEPE)<sup>2+</sup>(TCNQ)<sub>4</sub><sup>2-</sup>, 150-2,200  $\Omega^{-1} \text{ cm}^{-1}$ , are among the highest known for any organic material. They may be compared with the value of  $\sim 0.26 \Omega^{-1} \text{ cm}^{-1}$  reported<sup>8</sup> for polycrystalline 1,2-di(N-methyl-4-pyridinium)ethylene<sup>2+</sup>(TCNQ)<sub>2</sub><sup>2-</sup>, a closely related complex. The conductivity increases monotonically on cooling from 340 K to 30 mK (Fig. 1) with  $\sigma_{30 \text{ mK}}/\sigma_{RT} = 3.7$ . In the range 50-340 K,  $\sigma/\sigma_{RT} = 23T^{-0.55}$  with a weaker dependence at lower temperatures. In this region, a transition to superconductivity was not observed.





**Fig. 1** The normalised electrical conductivity of  $(\text{DEPE})^{2+}-(\text{TCNQ})_4^{2-}$  as a function of temperature. Insets: *a*, the low temperature region expanded and *b*, the crystal with current and voltage probes attached.

The temperature exponent of  $-0.55$  is considerably less than the values of  $-2.33$  for  $\text{TTF-TCNQ}^{9,3}$ , and  $-2.39$  for  $\text{HMTSF-TCNQ}^3$ . Allender *et al.*<sup>10</sup> have analysed the Fröhlich fluctuation-induced charge density wave conductivity  $\sigma_F$  of a one-dimensional metal obtaining the result  $\sigma_F \propto [T_c/(T-T_c)]^{0.5}$ , which, while it does not fit  $\text{TTF-TCNQ}$ , would fit the present data if  $T_c \ll T$ . If this theory holds (see also Strässler and Toombs<sup>11</sup>), we shall need to understand why the electron-acoustic phonon interactions would be dominant for our material, while optical phonon interactions must be involved for  $\text{TTF-TCNQ}^{12}$ . It is also interesting that the  $\sigma/\sigma_{RT}$  against  $T$  plot for  $(\text{DEPE})^{2+}-(\text{TCNQ})_4^{2-}$  down to 100 K closely follows that for neodymium, where the conductivity is dominated by spin disorder scattering<sup>13</sup>.

The anisotropy of conductivity has still to be established. If it turned out to be two-dimensional, as is found in a related semiconductor<sup>6</sup>, rather than one-dimensional, this might explain the absence of a Peierl's transition. The difference between the metallic and semiconducting species may depend on crystal structure, defect state, or indeed on differences in degree of charge transfer in the surface<sup>14,15</sup> on which we are now working.

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## Model reaction for biological reduction of nitrate involving $\text{Mo(III)/Mo(V)}$

WE have characterised the reduction of nitrate by a monomolecular molybdenum (III) species in aqueous solutions. Nitrate reduction is catalysed by nitrate reductases which are molybdoproteins<sup>1</sup>. Nitrate is reduced to nitrite by nitrate reductase, the nitrite being further reduced to ammonium ions by nitrite reductase which contains iron but no molybdenum<sup>2</sup>. Evidence indicates that nitrate reductases are monomolecular in molybdenum<sup>3,4</sup>. Therefore, a molybdenum-based model system for nitrate reductase should be monomolecular in molybdenum and undergo a change of oxidation state of two [ $\text{Mo(III)} \rightarrow \text{Mo(V)}$  or  $\text{Mo(IV)} \rightarrow \text{Mo(VI)}$ ].

Advances in the understanding of the chemistry of  $\text{Mo(III)}$ , (refs 5–8), the observation that  $\text{Mo(III)}$  reduces nitrate non-enzymatically<sup>9</sup> and the suggestions<sup>10,11</sup> that the  $\text{Mo(III)/Mo(V)}$  couple may be involved in certain enzymes containing molybdenum have led us to study the reaction between the hexaquo-molybdenum cation,  $\text{Mo(H}_2\text{O)}_6^{3+}$ , and nitrate.

The reaction between  $\text{Mo(H}_2\text{O)}_6^{3+}$  and nitrate was followed spectrophotometrically in 1 M *p*-toluene sulphonic acid (HPTS) in strict anaerobic conditions (argon). Representative spectra for both  $\text{Mo(H}_2\text{O)}_6^{3+}$  and nitrate are presented in Fig. 1. The absorption spectrum of nitrite in 1 M HPTS exhibits a multi-component band (vibrational fine structure) between 350 and 400 nm which is attributable to the  ${}^1\text{B}_1 \rightarrow {}^1\text{A}_1$  electronic transition ( $n \rightarrow \pi^*$ ). The  $\text{Mo(H}_2\text{O)}_6^{3+}$  in the same solvent had a low absorption at 293 nm, indicative of the purity of the preparation<sup>8</sup>. When solutions of  $\text{Mo(H}_2\text{O)}_6^{3+}$  ( $4.2\text{--}4.7 \times 10^{-4}\text{ M}$ ) were mixed with excess nitrate ( $1.33 \times 10^{-2}\text{ M}$ ) in pseudo first-order conditions and the reaction allowed to proceed to completion, nitrite absorption between 350 and 400 nm was concomitant with an increase in absorbance at 293 nm. The molybdenum species resulting from the oxidation by nitrate, or by air oxidation in independent experiments, had a spectrum between 340 and 280 nm identical to that of  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O)}_6^{2+}$ , as reported by Ardon and Pernick<sup>12</sup>.

The kinetics of the reaction between  $\text{Mo(H}_2\text{O)}_6^{3+}$  and nitrate were determined using five different preparations of  $\text{Mo(III)}$  (containing varying amounts of  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O)}_6^{2+}$ ) at  $28.5 \pm 0.5^\circ\text{C}$  in 1 M HPTS in the presence of  $1.33 \times 10^{-2}\text{ M}$  nitrate. The  $k_f[\text{Mo}_2\text{O}_4^{2+}]$  was found to be  $2.92 (\pm 0.27) \times 10^{-2}\text{ l mol}^{-1}\text{ s}^{-1}$ . Detectable nitrite was not produced from nitrate in identical conditions when  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O)}_6^{2+}$ —prepared either by the oxidation in air of  $\text{Mo(H}_2\text{O)}_6^{3+}$  or by the reduction of  $\text{Na}_2\text{MoO}_4$  by hydrazine—was substituted for  $\text{Mo(III)}$ . Moreover, the spectrum of  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O)}_6^{2+}$  in 1 M HPTS did not change during 1 h of observation either in the presence or absence of  $1.33 \times 10^{-2}\text{ M}$  nitrate. The reduction of nitrate to nitrite by  $\text{Mo(V)}$  in the presence of  $\text{Cl}^-$  in tartrate buffer<sup>13</sup> is approximately 10 times slower than the reduction of nitrate by  $\text{Mo(III)}$  reported here.

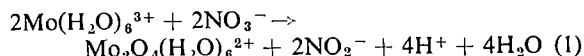
The concentration of the products of the reaction between  $\text{Mo(H}_2\text{O)}_6^{3+}$  and  $\text{NO}_3^-$  indicate the presence of one dimolybdenum species and 2 mol of nitrite (Table 1). The concentration of  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O)}_6^{2+}$  was measured spectrophotometrically, just before oxidation in air, and the concentration of the nitrite was subsequently determined colorimetrically<sup>14</sup>. Nitrite eventually disappeared from the solutions, suggesting that  $\text{Mo(V)}$  reduces nitrite as previously observed<sup>15</sup> in different conditions. When the reaction was allowed to go to completion, all of the  $\text{Mo(H}_2\text{O)}_6^{3+}$  present (the total molybdenum)

**Table 1** Stoichiometry of the oxidation of  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$  by nitrate

Experiment	Total Mo ( $\times 10^4\text{M}$ )	$[\text{NO}_2^-] (\times 10^4\text{M})$	$[\text{Mo}_2\text{O}_4^{2+}] (\times 10^4\text{M})$	Ratio $[\text{NO}_2^-]/[\text{Mo}_2\text{O}_4^{2+}]$
1	4.70	4.90	2.24	2.19
2	4.18	2.37	1.16	2.04
3	4.45	3.24	2.18	1.49
4	4.50	1.28	0.72	1.78
5	4.50	2.44	1.32	1.70

Nitrite measured colorimetrically,  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$  measured spectrophotometrically. Average ratio and standard deviation of  $[\text{NO}_2^-]/[\text{Mo}_2\text{O}_4^{2+}]$  was  $1.84 \pm 0.28$ .

was accounted for in the  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$  formed. So the overall reaction can be represented by:

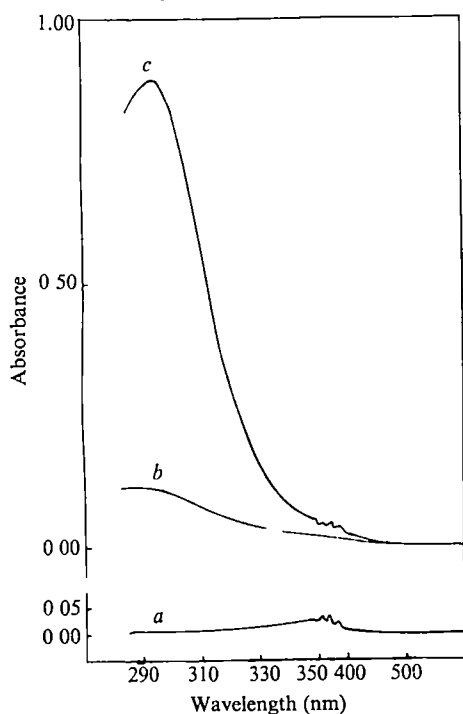


The stoichiometry was confirmed by the values obtained for the ratio  $k_f[\text{NO}_3^-]/k_f[\text{Mo}_2\text{O}_4^{2+}]$  (not shown). The nitrite concentration was measured during the reaction by adding sulphanic acid and N-1-naphthyl-ethylenediamine to the reaction mixture and recording the change in absorbance at 560 nm with time. The value of  $k_f[\text{NO}_3^-]$  (five independent determinations) was  $2.08 \pm 0.10$  times that of  $k_f[\text{Mo}_2\text{O}_4^{2+}]$ . The values for the  $k_f[\text{Mo}_2\text{O}_4^{2+}]$  were higher in the presence of the nitrite-trapping reagents, which may be attributable to their ability to pull the reaction to completion, and/or the effective removal of nitrite may prevent the further oxidation of  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$  produced in the reaction.

The reaction of nitrate with  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$  is approximately 10 times faster than the reaction reported by Guymon and Spence<sup>13</sup> for the reduction of nitrate by Mo(V). Moreover, the reaction of  $\text{K}_3\text{MoCl}_6$  with nitrate at pH 7.0 was too fast to measure by classical techniques, indicating that the reaction between Mo(III) and nitrate is faster at biological pH values than in the acid solutions investigated. We were unable to obtain kinetic data on the reaction between  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$  and nitrate at biological pH values because of the formation of molybdenum precipitates.

Nitrate reductases seem to contain one atom of molybdenum at each active centre. Preliminary EPR data demonstrate that

**Fig. 1** Spectra of a,  $\text{NO}_2^-$ ; b,  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ ; c,  $\text{Mo}_2\text{O}_4(\text{H}_2\text{O})_6^{2+}$  and  $\text{NO}_2^-$  produced by the oxidation of  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$  by  $\text{NO}_3^-$ . All spectra in 1 M HPTS.

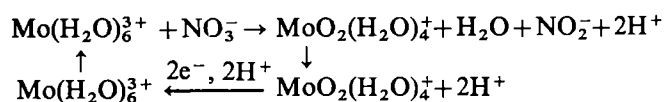


molybdenum can exist in the (V) oxidation state; one report<sup>18</sup> indicates that the Mo(V) state is the oxidised form of the enzyme, since the EPR signal disappears on reduction of the enzyme. Physiological substrates for nitrate reductases include  $\text{H}_2$ , NADPH,  $\text{H}^+$  and NADH,  $\text{H}^+$  (ref. 1). Therefore, a monomolecular molybdenum species in nitrate reductase undergoes a two-electron redox reaction and it is possible that Mo(V) is the oxidised molybdenum species.

A number of inorganic models for nitrate reductase involving the Mo(V)/Mo(VI) couple have been reported<sup>13,17-20</sup>. They all involve a single electron transfer and have the disadvantage that nitric oxide or a nitrogen (IV) oxide is formed as the reduced product. They represent models for enzymic nitrate reduction only if one postulates that molybdenum exists in a hydrophobic region of the enzyme. The nitrogen (IV) oxide formed in this hydrophobic region would undergo a disproportionation in the aqueous enzyme solvent resulting in the formation of nitrite and nitrate<sup>18</sup>.

On the other hand, the Mo(III)/Mo(V) model shown here involves an overall two electron transfer, could take place in either an aqueous or a hydrophobic (depending on the ligands involved in the molybdenum coordination) region of the enzyme, and produces nitrite as the reduced product and Mo(V) as the oxidised product. Moreover, the Mo(III)/Mo(V) couple is monomolecular in molybdenum; the dioxymolybdenum (V) species being formed in aqueous environments by the dimerisation of a presumed  $\text{MoO}_2^+(\text{V})$  species. These arguments suggest that the reduction of nitrate by  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$  is a valid model system for nitrate reductase.

Williams<sup>20</sup> has postulated an oxygen atom transfer mechanism to explain the biological reduction of nitrate which is reformulated in Fig. 2.



**Fig. 2** Postulated oxygen atom transfer mechanism for the enzymic reduction of nitrate by  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ , assuming NADPH,  $\text{H}^+$  to be the substrate for the reduction of  $\text{MoO}_2^+(\text{H}_2\text{O})_4$  to  $\text{Mo}(\text{H}_2\text{O})_6^{3+}$ . The net overall reaction is  $\text{NADPH}, \text{H}^+ + \text{NO}_3^- \rightarrow \text{NADP}^+ + \text{NO}_2^- + \text{H}_2\text{O}$ .

This mechanism is consistent with (although not proven by) our data. This scheme postulates that the potential of the enzymic Mo(III) is sufficiently negative to reduce nitrate. The  $\text{MoO}_2^+(\text{V})$  produced is reduced by the enzymic electron transport chain which donates 2 electrons and 2 protons. The reactions presented are compatible with the electron transport studies<sup>16,21,22</sup> on nitrate reductases, which position molybdenum at the terminal end of the electron transport chain and are consistent with the known physiological substrates which donate 2 electrons and 2 protons to the electron transport chain.

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## Relevance of oxygen ligands to reduction of ligating dinitrogen

We have reported<sup>1</sup> the reduction of dinitrogen, in *cis*-[M(N<sub>2</sub>)<sub>2</sub>-(PMe<sub>2</sub>Ph)<sub>4</sub>] (*A*, where M = Mo or W), to ammonia, on treatment with sulphuric acid in methanol at 20 °C. The reaction of the molybdenum complex is complete in 3–4 min but the yield (≤ 0.8 mol per Mo atom) is only about half that from the tungsten complex (≤ 1.8 mol per W atom), which requires about 0.5 h for complete reaction. We now find that ammonia is produced almost equally well, (1.7 mol per W atom), but much more slowly, from a methanol solution of *A*, (with M = W) either at the boil (3–4 h) or on irradiation for 30 h at 20 °C (2 × 150-W tungsten filament lamps); a boiling ethanol solution yields only 0.4 mol NH<sub>3</sub> per W atom. In contrast, *A*, (with M = Mo) gives very little ammonia (only 0.08 mol per Mo at best) in any of the above conditions; instead it evolves dinitrogen and dihydrogen.

It is not clear why the molybdenum complex produces so little ammonia from its reaction with methanol alone, in contrast to both its reaction in presence of sulphuric acid and the reactions of its tungsten analogue. One dinitrogen molecule is rapidly lost in all reactions of *A*, (M = W or Mo) leading to some intermediate which, in accord with the general chemistry of molybdenum and tungsten, should probably be more labile in the molybdenum series.

This greater lability may account for a more ready loss of the second dinitrogen ligand from molybdenum and the generally lower yields of ammonia. The greater lability may also be particularly damaging when the intermediate is a methanol- or methoxy-hydrido complex in boiling methanol rather than a sulphato- or other oxo-anion complex at 20 °C. It is, however, possible that the tungsten and molybdenum complexes yield ammonia by different chemical mechanisms<sup>2</sup>.

In both the tungsten and the molybdenum series of complexes, the protonation of N<sub>2</sub> to NH<sub>3</sub> in good yield seems to depend on the replacement of the tertiary phosphine ligands by ligands ligating through oxygen (for example, OH<sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, MeOH). All are replaced from the tungsten complexes, but perhaps only two from the molybdenum. Sulphate is the most effective oxo-anion tried, but others, including phosphate, are almost as good. Chloride and bromide are less effective (our unpublished results).

The importance of ligating oxygen probably lies in its effectiveness as a  $\pi$ -electron donor to early transition metals especially in higher oxidation states. The progressive replacement of the first dinitrogen molecule, which is a strong  $\pi$ -acceptor, and the tertiary phosphine ligands (weak or neutral  $\pi$ -acceptors) by the  $\pi$ -donor oxygen ligands progressively raises the d-electron energy levels on the metal atom, so that electrons pass from the metal to the second dinitrogen molecule as its protonation proceeds; possibly by a mechanism outlined in ref. 2. Chloride and bromide are less effective as  $\pi$  donors and

so lend themselves to the isolation of complexes containing dinitrogen in intermediate stages of reduction<sup>2</sup>.

Oxygen ligands are undoubtedly important in promoting the protonation and reduction of ligating dinitrogen to hydrazine or ammonia in suitable early transition metal (groups IV to VI) complexes. Whenever reasonable yields (for example, > 0.1 mol N<sub>2</sub>H<sub>4</sub> or > 0.2 mol NH<sub>3</sub> per transition metal atom) of nitrogen hydrides have been obtained by protonation of a dinitrogen complex, an oxygen ligand, or an oxygen-containing solvent, such as hydroxide<sup>3</sup>, catechol<sup>3</sup>, ether<sup>4</sup>, methanol-sulphuric acid<sup>1</sup>, propylene carbonate or *N*-methylpyrrolidone<sup>5</sup>, has been involved.

This observation raises the question as to whether, in nitrogenase, the molybdenum, assumed to be the active site, is in an oxygen environment. Chemical evidence seems to point in that direction because almost quantitative yields of hydrazine have been obtained from dinitrogen by its reaction with V(OH)<sub>2</sub> either in aqueous alkaline suspension or in solution with catechol<sup>3</sup>. This demonstrates that molecular nitrogen can interact with early transition metal ions in oxidation states accessible in aqueous solution, and presumably also in other oxygen or oxygen-nitrogen environments such as may ligate molybdenum in the enzyme.

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## Elevation of selenium levels in air by xerography

ROOMS in which xerography machines are used for document reproduction frequently have a strong, characteristic 'selenium' smell. It seemed likely that this is caused by selenium compounds released from the selenium plates or drums charged with high voltages in the photosensitising step of the photoelectric printing process. To test this hypothesis, we sampled the air in an unventilated room (48 m<sup>3</sup> in volume) housing a xerographic machine, on which some 450–475 copies were reproduced in intermittent use daily. The air was drawn by a small sampling pump at a rate of 1 foot<sup>3</sup> h<sup>-1</sup> through a fritted diffuser into 20 ml of 0.1 N NaOH for 9 h d<sup>-1</sup> for 4 d. The selenium trapped (0.005–0.015  $\mu$ g) was determined according to the fluorimetric method of Olson<sup>1</sup>.

Values obtained in replicate runs lay between 20 and 60 ng of selenium (as Se) per m<sup>3</sup> of air. The variation in levels may be ascribed to differences in the number of copies made and the non-compliance of users of the machine with a request to keep the door of the room closed during the sampling periods.

The levels found were thus invariably much higher than the 1.2 ng m<sup>-3</sup> measured in the San Francisco Bay area<sup>2</sup>, a level already considered to be elevated 2,400 times beyond the natural background accountable for in terms of soil and seawater contents. Average levels of 0.9 ng m<sup>-3</sup> Se were found in seven air samples taken in the Cambridge, Massachusetts area<sup>3</sup>.

Selenium is an essential trace element for bacteria and animals, and probably also for plants<sup>4</sup>; however, there seems to be a rather narrow range of beneficial selenium availability. Below certain levels, signs of selenium deficiency occur and at somewhat higher levels, signs of

selenium toxicity can become apparent<sup>5-7</sup>. Tolerable levels are influenced by environmental factors.

Whether elevated selenium levels in xerography rooms are hazardous remains to be seen, but avoidance of selenium buildup by good ventilation may well be indicated.

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## Do birds use waves for orientation when migrating across the sea?

MIGRATING birds apparently can determine their intended flight direction with reference to the Sun, the stars or the magnetic field<sup>1-3</sup>. This mechanism, however, cannot be used to prevent wind drift. We suggest that for this purpose they orientate themselves with reference to the waves when flying over the sea.

Low-flying, diurnal migrants have been found to compensate completely for wind drift over land<sup>4,5</sup>. Most radar studies have generally produced evidence of at least partial compensation in both nocturnal and diurnal migrants over sea as well as over land<sup>6-13</sup>, although some investigations indicate uncompensated wind drift<sup>14-17</sup>. Partial compensation has been interpreted in terms of 'pseudodrift'; that is complete compensation is thought to take place, but populations with different intended track directions migrate in different proportions in different wind conditions<sup>8,10</sup>.

The following means to achieve complete compensation have been proposed<sup>18</sup>: (1) continuous reference of the flight track to the landscape<sup>4,7</sup>, (2) reference to the wind in combination with initial or occasional reference to landscape<sup>9,10</sup>, (3) inertial recording of rectilinear and angular accelerations<sup>7,20</sup>.

Radar studies in southern Scandinavia of the diurnal migration of cranes *Grus grus*<sup>21</sup>, wood pigeons *Columba palumbus*<sup>22</sup> and redwings *Turdus iliacus*<sup>23</sup> have revealed incomplete compensation for wind drift over the sea, but complete compensation over land (no data were available for redwing migration over land). The variation of track directions over the sea was significantly less than would be expected if the birds drifted from fixed heading directions. Pseudodrift was observed for pigeons over land in southernmost Sweden as birds departing from different geographical areas flew in slightly different directions, and the proportion of pigeons arriving from the different areas varied with wind conditions. The drift effect was, however,

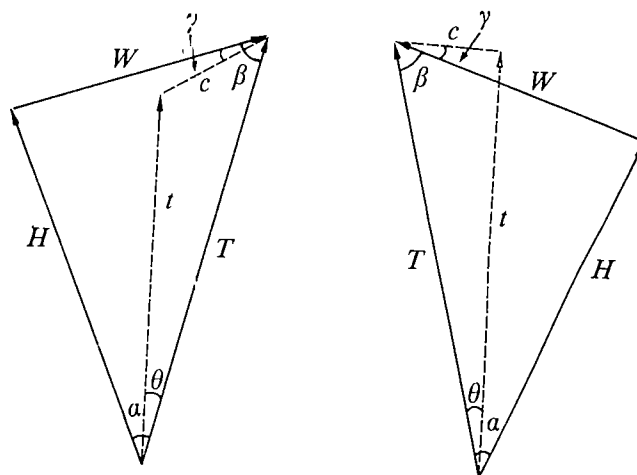


Fig. 1 Vector diagrams of bird movements in relation to wind and sea motion. The birds' ground speed and track direction ( $T$ ) are available from radar records, geostrophic wind speed and direction ( $W$ ) from ground synoptic maps. From these data, the birds' heading direction and true air speed ( $H$ ) can be calculated. If the birds drift because of the wave motion, the direction of velocity relative to the sea ( $t$ ) should be kept constant irrespective of wind to coincide with the intended track direction, and variation in direction of  $T$  should be due to variation in  $\theta$ .  $c$ , Velocity of waves;  $\gamma$ , angular difference between directions of wave movements and geostrophic winds. Dependent on whether winds blow from the left (left diagram) or right (right diagram) of the birds' track direction,  $\gamma$  should be subtracted from or added to  $\beta$  to determine the direction of  $c$ .  $\theta$  could be calculated from the equation.

$$\sin \theta = c \sin(\beta \pm \gamma) [c^2 + T^2 - 2cT \cos(\beta \pm \gamma)]^{-1/2}$$

significantly less over land than over the sea although the same birds were studied in both cases. Thus, pseudodrift cannot explain the increased amount of drift over the sea. Furthermore, these observations are at variance with the second and third (as far as pure inertial navigation is concerned) hypotheses.

In this report we present support for our hypothesis that the drift over sea was due to the birds using the wave pattern as 'landmarks'. It is reasonable to expect that the birds were not aware of the movement of the waves, but experienced the sea as a stationary landscape. We propose that the birds behaved in an analogous way over sea as over land, that is they projected their intended flight direction visually on to the wave pattern and headed into the wind to be able to fly along this course. The birds may determine the angle between their track direction and the waves by referring to irregularities, such as smaller or larger waves, of the wave landscape. (An inertial-Doppler navigating system would also be compatible with our hypothesis<sup>20</sup>.) Thus, according to our hypothesis, the birds compensated completely for wind drift in relation to the system of waves, and the resulting drift, as observed on the radar screen, was due to the motion of the sea relative to land. Orientation with reference to ocean waves has been suggested before<sup>18,24</sup>, but the consequence of drift relative to land has not been considered. This hypothesis may give a complete or partial (in combination with pseudodrift) explanation for drift over the sea, as witnessed in several studies<sup>8,16,11,13,16</sup>.

To test this hypothesis we used our data on crane, wood pigeon and redwing migration to calculate the angle between the

Table 1 Predicted amount of drift for birds migrating over the sea and orienting with reference to the wave pattern as compared with drift observed

	Fbo-L	Predicted Fbo-S	Fln-L	Fln-S	Observed $\pm 95\%$ confidence interval
Crane, Baltic Sea	0.40	0.51	0.29	0.41	0.25 $\pm$ 0.15
Wood pigeon, Baltic Sea	0.34	0.48	0.26	0.40	0.36 $\pm$ 0.17
Wood pigeon, Kattegatt	0.27	0.32	0.29	0.33	0.26 $\pm$ 0.21
Redwing, Baltic Sea	0.08	0.16	0.09	0.17	0.17 $\pm$ 0.16

Amount of drift is expressed as linear regression coefficients of  $\theta$  on  $\alpha$  (predictions) or of birds' track directions ( $T$ ) on  $\alpha$  (observations, see Fig. 1). Four predictions have been calculated on the basis of wave measurements at two different sites (Fbo and Fln) and of two different relationships between surface and geostrophic winds (see text).

**Table 2** Coefficients of linear regression of birds' track direction on the relative motion of the sea

Predicted		Observed $\pm 95\%$ confidence interval (correlation coefficient)			
		Fbo-L	Fbo-S	Fln-L	Fln-S
Crane, Baltic Sea	1	$0.54 \pm 0.28$ (0.65)	$0.48 \pm 0.26$ (0.64)	$0.74 \pm 0.39$ (0.65)	$0.59 \pm 0.33$ (0.64)
Wood pigeon, Baltic Sea	1	$0.98 \pm 0.48$ (0.67)	$0.75 \pm 0.35$ (0.69)	$1.33 \pm 0.64$ (0.68)	$0.93 \pm 0.43$ (0.69)
Wood pigeon, Kattegatt	1	$0.83 \pm 0.43$ (0.76)	$0.67 \pm 0.34$ (0.76)	$1.14 \pm 0.59$ (0.76)	$0.89 \pm 0.46$ (0.76)
Redwing, Baltic Sea	1	$1.87 \pm 1.45$ (0.70)	$0.93 \pm 0.71$ (0.70)	$1.99 \pm 1.50$ (0.71)	$1.04 \pm 0.77$ (0.71)

Linear regression coefficients of  $T$  on  $\theta$  (Fig. 1) are calculated (compare Fig. 2). Correlation coefficients are in parentheses. If birds fly on constant track directions relative to the sea, regression coefficients would be 1.

birds' track directions in relation to ground and sea, respectively, for each day of migration (Fig. 1) on the basis of the following assumptions.

(1) Waves move parallel to the surface wind direction. This assumption is of course violated in cases of rapid shifts of wind direction, but is a justifiable approximation since tides and currents are of no or little importance in the study areas<sup>25</sup>.

(2) Wave velocity ( $c$ ) was estimated from surface wind velocity on the basis of (a) measurements of wave periods in relation to winds at the lightships Falsterbo Rev (Fbo) in the southern Baltic Sea and Fladen (Fln) in the Kattegatt<sup>26</sup> (the wave period was calculated as a linear function of surface wind velocity in the interval  $3\text{--}14\text{ ms}^{-1}$ ) and (b) the theoretical relationship between wave period and velocity ( $c = p(g/2\pi)$ , where  $p$  is wave period,  $g$  is acceleration of gravity)<sup>27</sup>. The lightships were situated at sea areas with sufficient depths (30–50 m) for this factor not to impose any restrictions on wave propagation. The difference between wave movements with onshore and offshore winds (the lightships were situated 10–20 km offshore) was very small, and the combined sets of wave data were used. There was, however, a significant difference between wave movements at the two measuring stations, which cannot be explained. We therefore made separate calculations for the two stations.

(3) The birds travelled at altitudes of several hundred to a thousand metres. Direction and speed of the winds were evaluated from barometric pressure charts (geostrophic winds). Because of friction the surface wind force is reduced to about 70% of the geostrophic wind speed over the open sea (S) and 46% over land (L)<sup>28</sup>. Intermediate conditions would be expected in the study areas, due to the proximity of land, and we made separate calculations for both cases. On the basis of the above assumptions and calculations wave velocity was estimated from geostrophic wind velocity ( $W$ ) according to the equations:

$$\text{Fbo-L: } c = 3.32 + 0.111W; \quad \text{Fln-L: } c = 1.98 + 0.137W;$$

$$\text{Fbo-S: } c = 3.32 + 0.176W; \quad \text{Fln-S: } c = 1.98 + 0.219W.$$

These equations refer to average conditions determined from several thousands of observations of wave periods, and relationships would be expected to deviate at specific occasions, especially when the wind speed changes rapidly.

(4) Friction causes the surface wind direction to shift anticlockwise in relation to the geostrophic wind direction. The angle of deviation ( $\gamma$ , Fig. 1) is about  $14^\circ$  over open sea and  $38^\circ$  over land<sup>28</sup>.

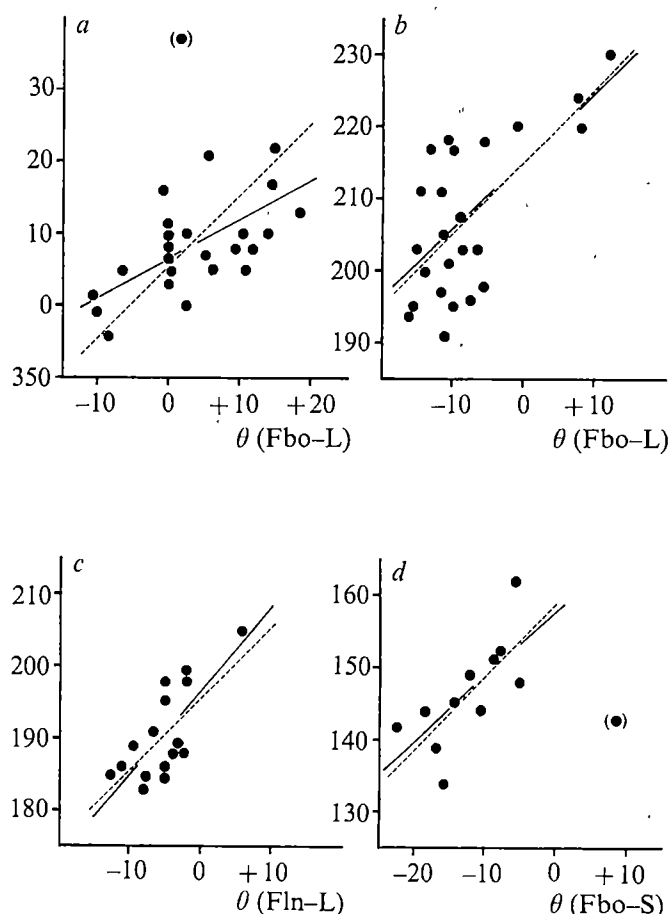
The amount of drift can be estimated on a scale from 0 (birds are flying on constant track directions) to 1 (fixed heading directions) if, by way of linear regression analysis, track directions are related to the angle between track and heading directions<sup>21–23</sup>. The amount of drift observed over sea varied between 0.17 and 0.36. These figures differ significantly from 0 as well as 1 and therefore lend no support to the hypotheses that the birds compensated completely for wind drift or that they drifted from fixed heading directions<sup>21–23</sup>. The observed amount of deflection is, however, close to what is predicted if the drift is due to the wave motion (Table 1).

If the birds are deflected due to the motion of the sea, the variation in observed track direction should be due to the variation of the angle between  $T$  and  $t$  as defined in Fig. 1. This

relationship is examined in Table 2 and Fig. 2.

Considering the uncertainties involved in applying data on the averaged relationship between waves and winds for specific instances, we think that the results form good support for the hypothesis that birds drift because of the motion of the sea.

The crossings over the southern Baltic Sea and the Kattegatt



**Fig. 2** Flight directions of birds ( $T$ ) during sea crossings in relation to the angle between track directions relative to ground and sea, respectively. The broken line (regression coefficient = 1) shows the relationship to be expected if the birds fly on fixed track directions in relation to the sea and drift by the motion of the sea. The solid line shows the linear least-squares fit to observational data (compare Fig. 1 and Table 2). One observation of crane migration is bracketed and excluded from the analysis. Mean track direction ( $037^\circ$ , only few flocks migrating) was considerably more towards the east than on any other day. Weather was different from all other occasions; strong west-south-west winds (approximately  $20\text{ ms}^{-1}$ ), low cloud ceiling, bad visibility and rain prevailed during the passage of a cyclone over southern Scandinavia. We suggest that the birds could not detect the sea, but drifted from a heading approximately pointing towards the preferred track direction ( $\alpha = 42^\circ$ ). Furthermore, data from 1 d of redwing migration are excluded, due to uncertainty about prevailing winds<sup>23</sup>. a, Crane, Baltic Sea; b, wood pigeon, Baltic Sea; c, wood pigeon, Kattegatt; d, redwing, Baltic Sea.

extend over fairly short distances (50–150 km), but cranes regularly cross much wider areas of open sea, such as the eastern Mediterranean, sometimes by night<sup>29</sup>, and the redwing, which typically migrates at night, regularly covers still longer distances over the open sea, for example, the North Sea from Norway to Britain and the Atlantic from Iceland to Britain<sup>24</sup>.

Without any points of reference for the track of flight, the birds may have to resort to heading towards the intended track direction and would consequently be drifted by the wind (or by clouds)<sup>30</sup>. By using landmarks or wave patterns they can completely (over land) or partly (over sea) compensate for the drift. Navigational ability may furnish birds with the possibility of reaching their goals in spite of drift over sea, by way of continually readjusting the intended track direction towards the goal.

It has been demonstrated that pigeons manage to home when released with frosted contact lenses (preventing detection of landmarks)<sup>31</sup> or over the sea with no sight of land<sup>32–34</sup>. In both cases, however, homing performance was worse, that is more pigeons were lost and loft re-entry times were longer, than for control birds released over land. The poor homing performance may be due to drift by winds and waves, respectively.

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## Oscillations of tropical insect populations

SAUNDERS AND BAZIN<sup>1</sup> have suggested that “only ecosystems with relatively few species can have a sufficiently high linear connectivity to produce oscillations without becoming unstable, which would explain why it is that population oscillations are observed in the Arctic but not in the

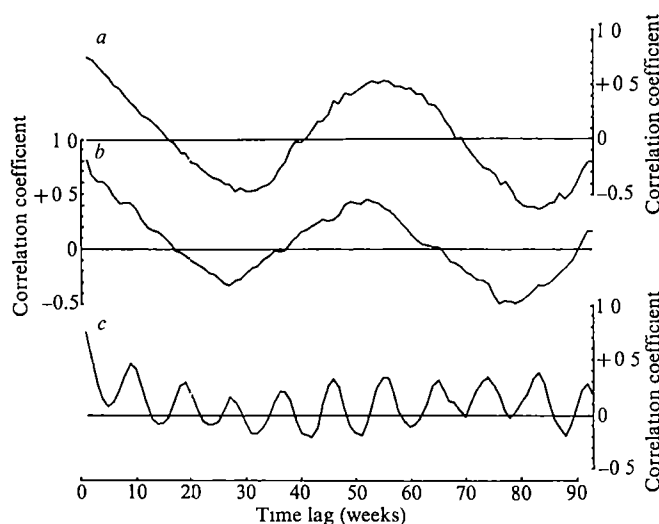
tropics”. Most numerical studies of insect populations in the tropics have been relatively short but what longer time series there are do not bear out this statement. The more favourable temperature conditions in the tropics can allow breeding to continue throughout the year, and typically 8–10 generations may be completed each year.

Nevertheless, annual cycles of abundance are commonplace and, not infrequently, shorter term oscillations with a period equal to the generation length are evident, they must arise through the delay caused by the insect passing through its developmental stages. These ‘generation’ cycles are remarkably persistent and do not damp out with time as predicted by Lotka<sup>2</sup>. They are particularly strong in suction trap records of the coffee leaf-miners, *Leucoptera meyricki* (Guesq.) (Lepidoptera, Lyonetiidae) and *L. coffeina* (Washb.) in Tanzania<sup>3</sup>, and are observed in populations of *Planococcoides njalensis* (Laing) and *Planococcus citri* (Risso) on cocoa in Ghana. Furthermore, the idea that the greater complexity of tropical ecosystems necessarily confers on them a higher degree of stability and thus that oscillations tend to damp out seems to be largely illusory.

Of all tropical tree crops, cocoa maintains one of the most complex insect ecosystems. On a world-wide basis Entwistle<sup>4</sup> has recorded more than 2,000 associated insects, and in Ghana some 250 ant species have been recorded from cocoa. The crop is grown under thinned forest shade and is maintained in its early years with the minimum of disturbance to the underbush vegetation which persists until the cocoa canopy becomes sufficiently thick to shade it out. It is thus in intimate contact with an overstorey of forest trees and an understorey of ground vegetation, and receives its insect fauna from both sources. In particular, the ant regimes which make up the bulk of the insect biomass on cocoa seem to be superimposed on the crop from the overstorey trees. The interrelationships between the components of the cocoa ecosystem are very complicated, involving homopteran–ant mutualistic associations and territorial antagonism between the more aggressive ant species, which leads to the formation of discrete, three-dimensional ant mosaics<sup>5</sup>. Agents such as the cocoa mirids and the mealybug-transmitted, cocoa-swollen shoot virus can lead to the large scale degeneration of the cocoa canopy with a consequent impact on the other elements of the ecosystem.

Yet in spite of the complexity of the cocoa ecosystem

Fig. 1 Serial correlations of a time series comprising 186 weekly records of the percentage of trees infested by three different insect species on a block of 238 Amazon cocoa trees. a, *Steatococcus* sp.; b, *Pheidole megacephala* (F.); c, *Phenacoccus hargreavesi* (Laing).





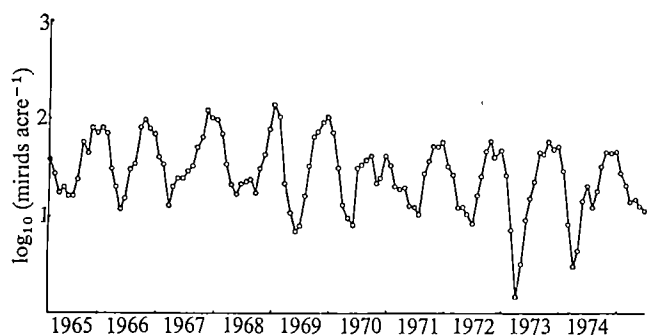


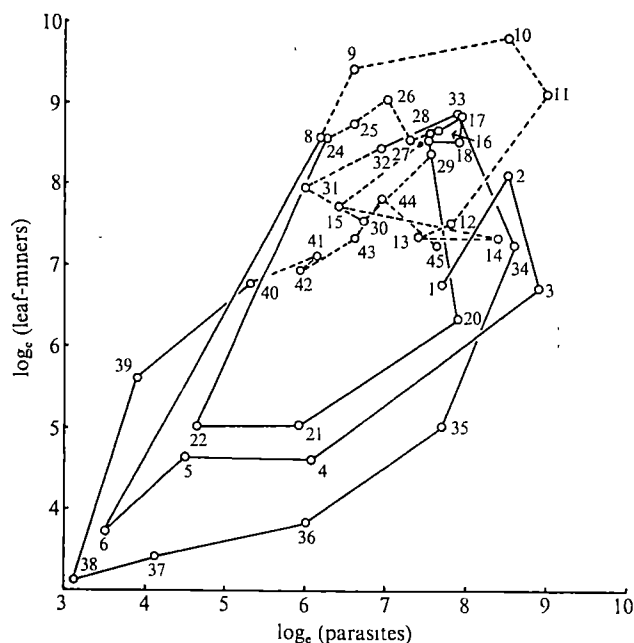
Fig. 2 Monthly assessment on 25 farms in eastern Ghana of populations of the cocoa mirids *Distantiella theobroma* (Dist.) and *Sahlbergella singularis* (Hagl.). The counts of the two species have been combined.

many of its component populations oscillate both numerically and spatially. Figure 1 shows three correlograms obtained from a serial correlation of time series of 186 weekly records of the percentage of trees infested on a block of 238 Amazon cocoa trees. *Steatococcus* sp. (Hemiptera, Coccidae) and *Pheidole megacephala* (F.) (Hemiptera, Formicidae) show particularly strong oscillations with a period of about 1 yr, and *Phenacoccus hargreavesi* (Laing) (Hemiptera, Coccidae) shows a cycle with a period of 9.2 weeks.

Population counts of the cocoa mirids, *Sahlbergella singularis* (Hagl.) and *Distantiella theobroma* (Dist.) made at monthly intervals on 25 farms in eastern Ghana since 1965 (Fig. 2) showed an annual cycle in abundance, and perhaps a much longer term cycle.

In Tanzania, daily catches in suction traps of *Leucoptera meyricki* and *L. coffeina* on arabica coffee were made at Lyamungu, on the southern slopes of Mount Kilimanjaro between 1960 and 1968, covering in all 62 moth generations<sup>3,6</sup>, and for the last 45 generations independent estimates have been made of the density of the larvae of the two species and their associated parasites (Fig. 3). It is clear that a 16-generation (two-year) cycle is superimposed on an 8-generation (one-year) cycle. A clearer picture of

Fig. 3 Number per generation per 100 leaves of larvae of coffee leaf-miners and their larval parasites on arabica coffee in Tanzania. Generations are numbered consecutively, solid and dashed lines indicating alternate years. No counts were made in generations 7 or 23.



the perturbations of the combined host-parasite system about its mean level can be obtained by a principle components analysis of logarithmically transformed values of hosts and parasites since values along the first components axis are a linear combination of the host and parasite scores (Fig. 4). Here again the two-year cycle is very evident. Population counts of the two leaf-miner species were made at Lyamungu by Notley<sup>7,8</sup> between 1938 and 1940, and a similar two-year cycle is discernible in his data.

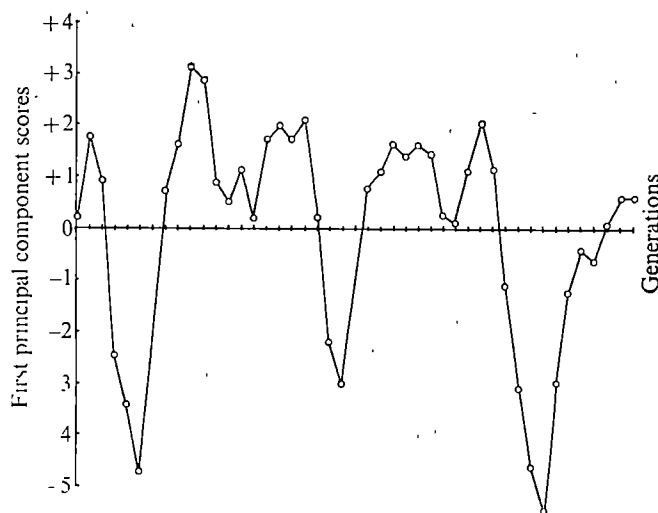


Fig. 4 Displacement from equilibrium of the leaf-miner-parasite system of Fig. 3 considered as a whole and expressed as distances along the first principal component axis from a principal component analysis of the logarithmically transformed numbers of leaf-miners and parasites per generation.

It seems likely that the annual leaf-miner cycle is driven by seasonal weather changes and is not caused by interactions within the system, but it is not at present obvious how an annual weather pattern could interact with the system to produce a two-year cycle. There is some evidence that the leaf-miner parasites do no more than follow the cycles of leaf-miners.

It could be argued that the lynx cycle, to which Saunders and Bazin<sup>1</sup> are presumably alluding in their reference to the Arctic, is not comparable to the leaf-miner cycle because of the great difference in periodic lengths but when the two cycles are compared in terms of generation length this apparent difference disappears. The driving force behind both cycles is obscure. Bulmer<sup>9</sup>, has suggested that the cycles of Arctic predators are caused directly or indirectly by the cycle in the snowshoe rabbit but this leaves unanswered the question of why the snowshoe rabbit population should be cyclical.

Williamson<sup>10</sup> has suggested that the standard deviation of the  $\log_{10}$  generation values can be used as a measure of population variability and thus of the amplitude of oscillations, and he quotes a number of examples. The standard deviation of the leaf-miner population is 0.768, which compares with a standard deviation of 0.558 for the lynx cycle. The leaf-miner standard deviation is similar to many of the European insects cited by Williamson<sup>10</sup> and compares with a value of 0.771 observed for the black headed budworm *Acleris variaria* (Fern) and 0.595 for the European spruce sawfly *Diprion hercyniae* (Htg) in Canada, calculated from data given by Morris<sup>11</sup>.

It is clear that not only do tropical insect populations show cyclical behaviour, but also that the amplitude of the cycles is comparable to those of temperate insects. Furthermore, even in the cocoa ecosystem, which is considerably complex and has many features in common with tropical forests, population cycling is observed in many species.

Tropical insect host-predator systems are worthy of much

closer examination if only because they have the advantage over temperate systems of a compression of the time scale by a factor of 8 to 10.

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## Divergent X-ray-induced mutation rates in the mouse for *H* and "7-locus" groups of loci

INCREASED demand for nuclear power re-emphasises the need for a well-grounded estimate of the genetic risk of environmental mutagens in general and of ionising radiations in particular. With respect to germ-line, specific-locus mutation rates, estimates for man have been based primarily on the mouse 7-locus test, carried out on a large scale at Oak Ridge National Laboratory (USA) and Harwell (UK). Among the many important radiobiological findings recently summarised<sup>1</sup> are the following: the mutation rate depends on sex, on the type of germ cell irradiated, on radiation quality (X rays, neutrons), on dose rate (rad min<sup>-1</sup>), on fractionation (number of treatments during a certain period) and on total dose.

With this information in hand, we can inquire about the limitations inherent in the 7-locus test. First, is the sample of seven loci (*a*, *b*, *c<sup>h</sup>*, *p*, *d*, *se*, *s*) representative of the entire mouse genome? Second, does the test, detecting so far only the change from dominant to recessive (it can also detect wild type to dominant), adequately approximate the rates for other types of specific-locus mutational change? Two reports bear directly on these questions.

Lyon and Morris<sup>2</sup>, using 7-locus test methodology, tested five other loci together with one of the original seven (*a*, *bp<sup>H</sup>*, *fz*, *ln*, *pa*, *pe<sup>H</sup>*) in a "6-locus" test. Dominant-type males were mated to untreated females which were homozygous for recessive alleles at the loci of interest. Mutants were detected in the F<sub>1</sub> progeny by their visible recessive phenotypes, that is, by a loss of dominant allele activity. After 600 rad of X rays, the mean spermatogonial mutation rate was one-third that of the 7-locus mean.

Bailey and Kohn<sup>3</sup> screened a different and much larger (>30) set of loci (histocompatibility or *H*) whose alleles are codominant. The *H* test detected losses in antigenicity (analogous to the loss of the dominant phenotype in the 7-locus test), and also a change or gain in antigenicity, which might signal a change in one or more codons or the appearance of a new active gene. The result of their experiment with a single dose of 522 rad suggested that the *H* loci were less sensitive than the "7 loci", but did not establish the size of the difference.

We have therefore made a quantitative comparison. We used a standardised *H*-test procedure<sup>3-5</sup> in which tail-skin grafts were orthotopically exchanged in a "reciprocal circle" among syngeneic mice, 37-43 d old. Each mouse provided one graft for each of two other mice of similar sex and received two grafts in return. All grafts should have been accepted. Suspected mutants, detected on the basis of

**Table 1** Predicted number of mutants per dose group based on the results with the 7-locus test

Dose (rad)	H tested F <sub>1</sub> s	K (× 10 <sup>-7</sup> )	Predicted nos of induced mutants
Controls	2,335	—	—
Single doses			
350	1,239	2.8	3.6
500	947	2.5	3.6
650	1,285	2.2	5.5
800	1,603	1.65	6.3
Split, 1 d			
500 (150+350)	1,107	3.35	5.6
650 (300+350)	1,339	3.7	9.7
800 (300+500)	1,061	4.1	10.4
Split, 5 or 8 weeks			
650 (300+350)	1,422	2.8	7.8
800 (300+500)	1,276	2.6	8.0
Total treated	11,279	—	60.5

The predicted number of induced mutants was equal to: *H* loci tested per irradiated gamete (30) × dose (rad) × animals tested × *K*. *K* (expected rate of induction of mutations per locus per rad) was calculated separately for each of our dose groups on the basis of the Oak Ridge and Harwell statistics for the 7-locus test, given in Tables VI and VII of ref. 1. The doses there are in r.; we used them as equivalent to rad. For single exposures, the value of *K* was read from a four-point curve over the range 300-900 r. For split doses 1 d apart, *K* was read from a line determined by two points, that for a single dose of 300 r. and that for 1,000 r. given in two 500 r. fractions. This made the conservative assumption that splitting 300 r. did not increase *K*, but that such split doses become relatively more effective as the total split-dose rose to 1,000 r. For split doses 5 or 8 weeks apart (mean 52 d), no effect of fractionation was assumed; *K* for each fraction was that read from the single-dose curve. The values for the two fractions were combined to give the single equivalent value of the table.

their pattern of graft rejection 57-63 d after grafting, were backcrossed to establish transmission of mutations.

The grafted animals were the F<sub>1</sub> progeny of pretested C males (BALB/cKh) whose pelvic region had been X rayed at 105-152 d old, and who were mated 75 d later to pretested B6 females (C57BL/6Kh). The radiation factors were 250 kVcp X rays, half value layer 1.2 mm Cu; intensity, 60-65 rad min<sup>-1</sup>. We used 34 control and 214 irradiated males, who were mated with 692 B6 females. The males were kept in service for 1-1.5 yr after initiation of mating, and were mated with one female at a time.

Two classes of loci were at risk<sup>6,7</sup>. Class I loci were heterozygous in the F<sub>1</sub> animal because the B6 and C parents carried different alleles. A minimum of 30 such loci was irradiated in the C male and tested for spermatogonial mutations in the BC6F<sub>1</sub> progeny. Class II loci were homozygous and will be dealt with in a later report. Results with class II loci are consistent with those for class I.

In all, 11,279 F<sub>1</sub>s were tested for the effects of single and split spermatogonial doses of 350-800 rad. There were 2,335 controls. Results with the 7-locus test predicted that the number of induced mutants would be 60 (Table 1). We observed only one class I, C-allele mutant in the treated groups. It was of the "gain and loss" type (a specificity was lost and was replaced by a new one); its father had received 650 rad (300+350, 1 d apart). Four other class I mutations were detected (three gain and loss, and one loss), but had occurred in B6 alleles, that is, in the unirradiated maternal gamete. No class I mutants were detected in the controls. Failure to find mutants was not considered to be a failure to detect them, as more than 60 class I and II mutants in 30,000 mice have been reported<sup>3,6</sup>.

The results for the mouse with the 7-locus, 6-locus and *H* tests show that different specific-locus tests applied to the same germ cell (spermatogonium) yielded different X-ray-induced mutation rates (relative values of 60, 20 and 1, respectively). This raises the question, to what extent can any particular set of loci represent the mammalian genome in this field? The results with the *H* loci, of course, might

indicate unique factors in histocompatibility radiation genetics that do not apply elsewhere. In any event, it appears that further research with various loci is needed.

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## Specific marker chromosome 14 in malignant lymphomas

A CONSISTENT structural abnormality of chromosome 14—an extra band at the terminal of the long arm—has been found in 10 out of 12 African Burkitt lymphomas<sup>1</sup>. We report here a similar long acrocentric marker chromosome derived from chromosome 14 in other types of malignant lymphoma.

The samples used were seven lymphosarcomas, three reticulum cell sarcomas, six Hodgkin's lymphomas and one follicular lymphoma. The lymphoma cells from lymph nodes, peripheral blood, bone marrow, ascites, pleural effusion and a cell line *in vitro* were cultured for 24 h in RPMI 1640 medium with 10% foetal calf serum, and the chromosome specimens were prepared as usual and banded with Giemsa<sup>2</sup>. As controls, lymph node cells from three cases with chronic lymphadenitis were examined.

Table 1 summarises the results obtained. Many cells from various lymphoma cases reveal a long acrocentric marker chromosome. This marker was found in three out

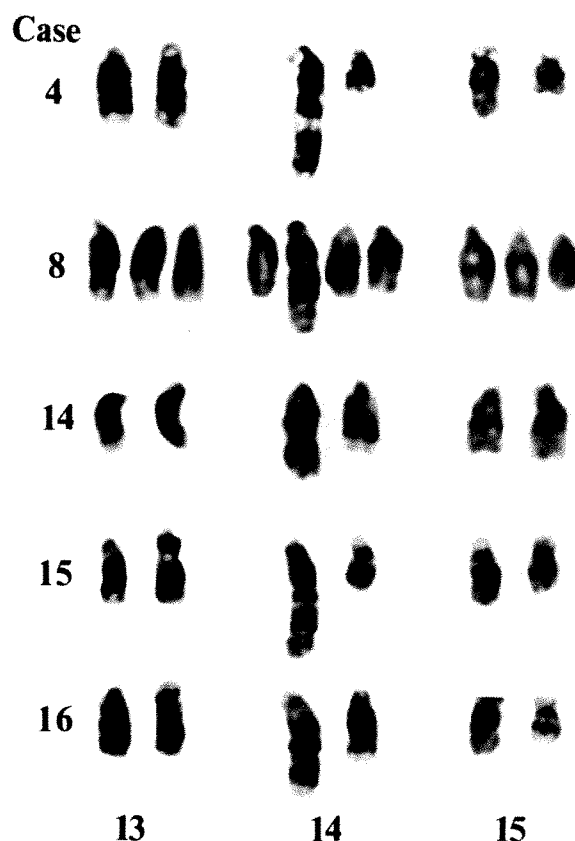


Fig. 1 Partial karyotype of D-group chromosomes of five mitotic malignant lymphoma cells banded with Giemsa. Note a longer chromosome 14 than usual in each case.

of four cases of lymphoblastic lymphosarcoma and absent in three lymphocytic lymphosarcomas. About 50% of the hypotetraploid cells of reticulum cell sarcoma in cases 8 and 9 had a similar marker although in case 10, examined after irradiation, this marker was not frequent. A similar acrocentric marker was found in three out of six cases of Hodgkin's disease. Cells with this marker were hyperdiploid in case 14, and near triploid in case 15. Both hypodiploid and near triploid cells in case 16 had this abnormal chromosome. Cases 11, 12 and 13 which were in a less advanced histological stage lacked this acrocentric marker. Abnormal

Table 1 Incidence of long acrocentric marker chromosome in malignant lymphoma cells

Case no.	Diagnosis	Total cells counted	Incidence (%)	$\frac{\text{Cells with the marker}}{\text{Total cells counted}} \times 100$
1	LSaL (lymphoblastic)	58	0	
2	LSaL (lymphoblastic)	46	19	(65% of hyperdiploid cells)
3	LSaL (lymphoblastic)	31	30	(all hyperdiploid cells)
4	LSaL (lymphoblastic)	50	100	(all near diploid cells)
5	LSaL (lymphocytic)	27	0	
6	LSa (lymphocytic)	28	0	
7	LSa (lymphocytic)	30	0	
8	RCSa	28	54	(60% of hypotetraploid cells)
9	RCSa	40	45	(50% of hypotetraploid cells)
10	RCSa	50	20	(22% of hypotetraploid cells)
11	HD (LP)	68	0	
12	HD (LP)	50	0	
13	HD (MC)	69	0	
14	HD (MC)	50	98	(all hyperdiploid cells)
15	HD (LD)	50	78	(all near triploid cells)
16	*Hodgkin cell line	50	100	(all hypodiploid and near triploid cells)
17	FL	58	0	
18	Chronic lymphadenitis	33	0	
19	Chronic lymphadenitis	10	0	
20	Chronic lymphadenitis	13	0	

LSa (L), lymphosarcoma (leukaemic); HD, Hodgkin's disease; RCSa, reticulum cell sarcoma; FL, follicular lymphoma; LP, lymphocytic predominance; MC, mixed cellularity; LD, lymphocytic depletion.

\*Derived from pleural effusion of Hodgkin sarcoma. Chromosome analysis was done at the 50th transfer generation (350 d).

chromosomes were not found in the follicular lymphoma and the three chronic lymphadenitis.

Detailed analysis revealed that the marker was an abnormal chromosome 14 with a longer arm than usual, representing a tandem 14:14 long arm translocation (Fig. 1). Although some other chromosomal abnormalities were also found in the malignant lymphomas examined, none was common as the acrocentric marker involving chromosome 14.

The specific involvement of chromosome 14 in clonally proliferating human lymphocytes has been also demonstrated in ataxia-telangiectasia<sup>3,4</sup>. The above findings therefore support the hypothesis by McCaw *et al.*<sup>4</sup> that structural rearrangement of the long arm of chromosome 14 may be a step towards the development of lymphoid malignancies.

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## Examination of ATCC stocks for HeLa marker chromosomes in human cell lines

INTRASPECIES contamination of cell cultures is a serious technical problem when more than one cell line is maintained in continuous serial subculture within a laboratory. Biochemical<sup>1-3</sup> and immunological<sup>4,5</sup> evidence has strongly suggested that many "cell lines" that are commonly used in the laboratory around the world have been contaminated with HeLa cells. Chromosome rearrangements resulting in specific "markers" provide a unique opportunity to monitor for intralinear specificity in HeLa and other human cells. Because certain cell lines in the American Type Culture Collection (ATCC) repository have already been shown to possess the type A (fast) G6PD characteristic of HeLa, we initiated a survey of these cell lines for the chromosome features described for HeLa cells<sup>6-9</sup> using the trypsin-Giemsa banding technique<sup>10</sup>. Ten cell lines, including two HeLa lines, have been studied so far. Five of these lines have been found to contain marker chromosomes typical of HeLa cells. The non-HeLa cell lines have chromosome characteristics of their own which can also be used to monitor their purity.

The HeLa S3, ATCC-CCL 2.2 and HeLa, ATCC-CCL 2, have four distinct marker chromosomes (Figs 1a and 2). Marker 1 (M1) is formed by the fusion of centromere and the long arm of chromosome 1 and the long arm of chromosome 3. Marker 2 (M2) consists of the short arm of chromosome 3 and the long arm of chromosome 5. Marker 3 (M3) is an isochromosome of the short arm of chromosome 5, and usually occurs in more than one copy. Marker 4 (M4) consists of the long arm of chromosome 11 and an arm of chromosome 19. Our views on the origin of M4 differ from those of other investigators<sup>6-8,11,12</sup>.

Marker chromosomes typical of HeLa cells (HM) are

found in the following cell lines: Detroit-6, ATCC-CCL 3; Chang liver, ATCC-CCL 13; KB, ATCC-CCL 17; AV<sub>3</sub>, ATCC-CCL 21 and FL, ATCC-CCL 62. These lines are all epithelial in general morphology and possess the fast migrating variant (type A) of the isoenzyme glucose-6-phosphate dehydrogenase (G6PD). Detroit-6 cells lack HM1 but exhibit two copies of HM2, three or four copies of HM3, and HM4 is found occasionally. Even though this cell line was reported to have originated from a male Caucasian<sup>13</sup>, quinacrine fluorescence studies did not reveal the presence of the Y chromosome. In addition, there is a large isochromosome (LI) formed by the duplication of the long arm of chromosome 10 (Fig. 2). The Chang liver line exhibits all four HeLa-like markers, two copies of HM1, one copy of HM2, four copies of HM3 and a single copy of HM4 (Fig. 2). In the KB line, reported to have originated from a male Caucasian<sup>14</sup>, HM1 is missing but one copy of HM2, four copies of HM3 and a single copy of HM4 are present. There is a large submetacentric (LSC) marker chromosome probably formed by the translocation between an arm of chromosome no. 3 and the long arm of chromosome 7 (Figs 1c and 2). Quinacrine fluorescence studies also revealed the absence of the Y chromosome in this line. The AV<sub>3</sub> cell line does not reveal the presence of HM1 or HM2, however, three copies of HM3 and one copy of HM4 are noted. A large submetacentric chromosome (LSC), similar to the one found in KB is present (Fig. 2). All four marker chromosomes typical of HeLa cells are found in the FL line. Three copies of HM3 and two copies of HM4 are always present. In addition, these cells possess one large submetacentric chromosome, two large isochromes and one medium-sized isochromosome. The large submetacentric chromosome (LSC) is formed by chromosome 2 with a terminal duplication of the long arm. The large isochromosome (LI 1) is formed by the duplication of the long arm of chromosome 7 and the second large isochromosome (LI 2) is formed by the duplication of the long arm of chromosome 9. The medium-sized isochromosome is formed by the duplication of the long arm of chromosome 14 (Figs 1b and 2).

Marker chromosomes typical of HeLa cells are not found in the following cell lines: RPMI 2650, ATCC-CCL 30 (Fig. 1d); RD, ATCC-CCL 136 and Detroit 562, ATCC-CCL 138. These lines also have an epithelial morphology but possess the slow migrating variant (type B) of G6PD. The RPMI 2650 cells possess the Y chromosome as determined by the quinacrine fluorescence technique. Examination of RD and Detroit 562 lines using the same technique did not reveal the Y chromosome which would be expected since they are of female origin. Detroit 562 cells contain a large submetacentric marker chromosome probably formed by chromosome 5 with a terminal duplication of the long arm.

Table 1 summarises our findings. The absence of the Y chromosome, possession of G6PD type A and the presence of HeLa marker chromosomes provide strong evidence that Detroit-6 and KB are cross contaminated with HeLa cells. Although the donor sex and racial origin of the Chang liver and FL are unknown, the fact that they both possess all four HeLa marker chromosomes indicates the possibility that these lines also are cross contaminated with HeLa cells. The AV<sub>3</sub> which is of negro origin and donor sex unknown, likewise possesses HeLa marker chromosomes. The absence of certain HeLa marker chromosomes in some of these cell lines may be due to partial contamination with HeLa cells probably through somatic cell hybridisation. The other possibility is that the gain or loss of these markers, after their initial appearance in the HeLa-contaminated parental stock, could represent no more than random effects of different culture conditions in different laboratories. The occurrence of identical large submetacentric chromosomes (LSC) in both the KB and



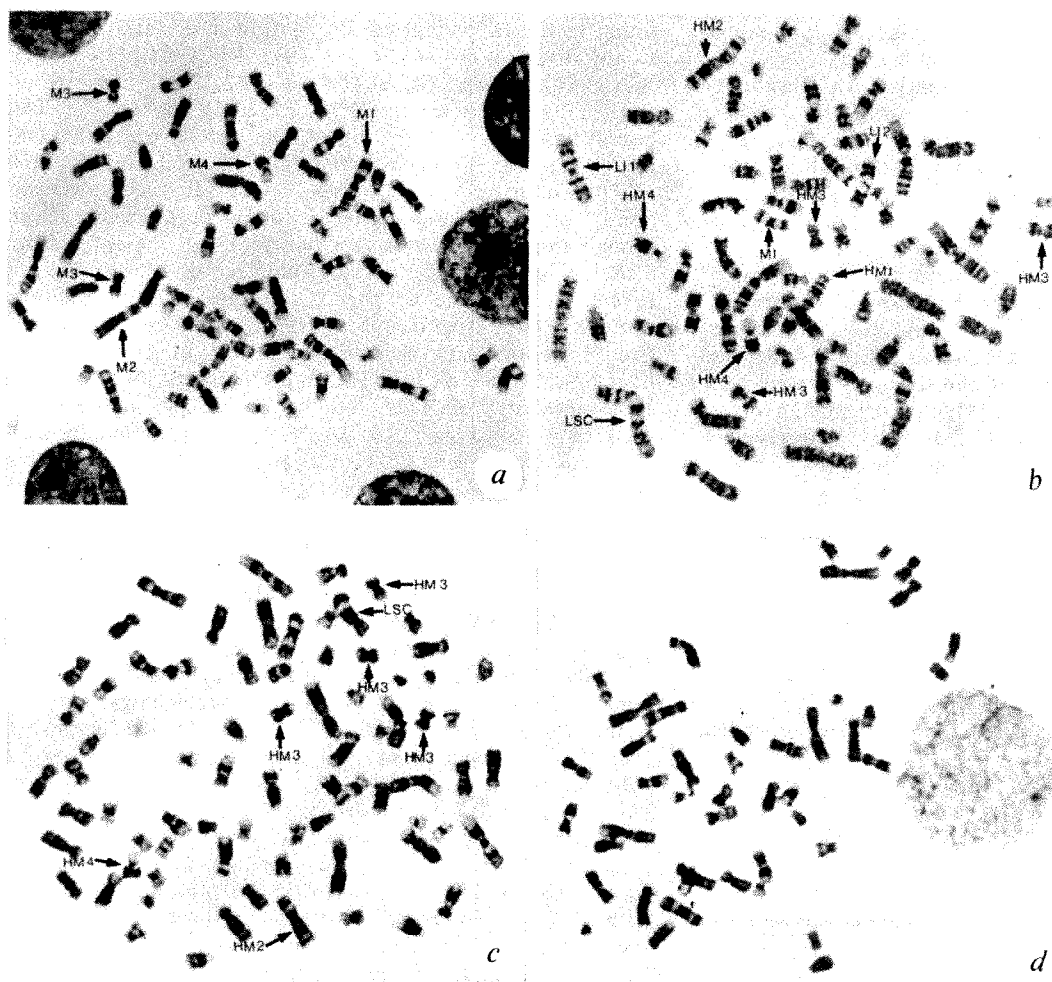


Fig. 1 *a*, Trypsin-Giemsa banded metaphase chromosomes of HeLa, S3, ATCC-CCL 2.2. Arrows indicate marker (M) chromosomes. *b*, Trypsin-Giemsa banded metaphase chromosomes of FL, ATCC-CCL 62. Arrows indicate HeLa-like markers (HM), large and medium-sized isochromosomes (LI 1, LI 2 and MI) and a large submetacentric chromosome (LSC). *c*, Trypsin-Giemsa banded metaphase chromosomes of KB, ATCC-CCL 17. Arrows indicate HeLa-like markers (HM) and a large submetacentric chromosome (LSC). *d*, Trypsin-Giemsa banded metaphase chromosomes of RPMI 2650, ATCC-CCL 30. Note the lack of HeLa-like marker (HM) chromosomes.

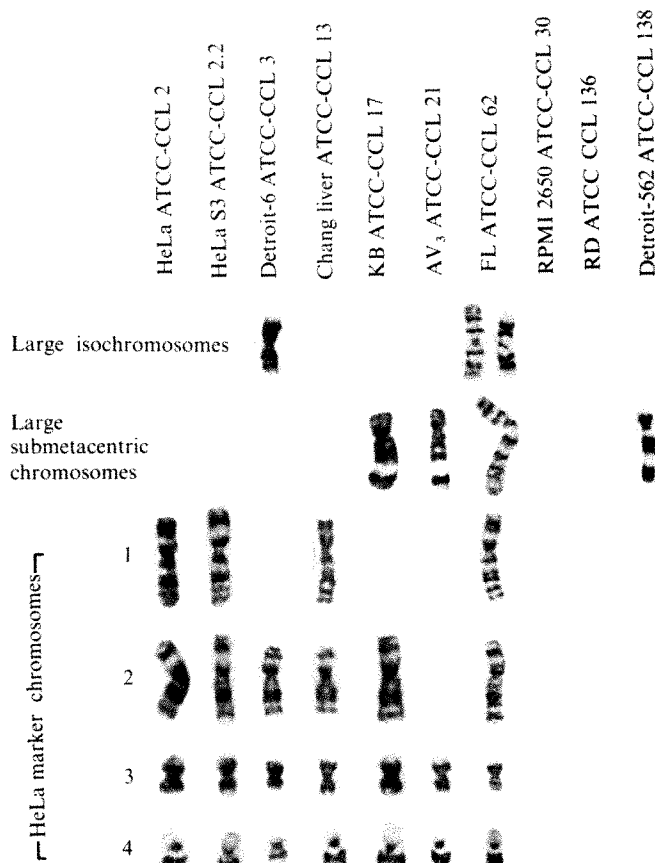
AV<sub>3</sub> indicates that these two cell lines might have been cross contaminated with each other or that these two identical markers arose independently of each other. At the time when these certified cell lines were deposited in the ATCC Animal Cell Repository as frozen ampoules, technology was not available to check for intraspecific cell contamination. With modern techniques it is now possible to monitor cell lines for cross contamination of this kind. Since we have examined these lines carefully within two passages beyond that of the frozen seed stock, using trypsin-Giemsa banded marker chromosomes, these lines were undoubtedly already contaminated with HeLa cells before reaching the ATCC Repository. On the other hand, lack of HeLa-like markers coupled with the findings for G6PD and the Y chromosome clearly indicate that RPMI 2650, RD and Detroit 562 are not cross contaminated with HeLa cells (Table 1).

Thus it seems that investigators who culture more than one cell line in the laboratory at the same time run a high risk of cross contaminating their cultures. Consequently, all cell lines should be periodically and frequently monitored through isoenzyme analysis, chromosome banding and immunological techniques to prevent intra- or interspecies contamination.

*Note added in proof:* The above information is already incorporated in a new edition of the ATCC catalogue (ATCC Catalogue of Strains II, first edition, 1975). A discussion of the intraspecific cell contamination problem appears in the introduction and all of the ATCC stocks which are known to bear HeLa-like characteristics are clearly marked in the catalogue. We are currently examining all other ATCC stock of human cell lines for chromosome features characteristic of HeLa cells and the information will be made available in subsequent publications.

Since the submission of this manuscript for publication

Fig. 2 Trypsin-Giemsa banded marker chromosomes of cell lines examined.



**Table 1** ATCC stocks of human cell lines examined for HeLa marker chromosomes

Cell Line	Deposited in ATCC Repository Date	Passage no.	Tissue of origin	Racial origin	Type of G6PD	Sex of donor	Y chromo- some	Passage no.	Chromosome no. Mode	Range	Marker chromosomes HeLa markers					Other markers
											No. 1	No. 2	No. 3	No. 4		
HeLa (ATCC-CCL 2)	6-19-63	?	Cervical carcinoma	Negro	A	F	—	?	84	58-179	+	+	4-5*	2*	—	
HeLa S3 (ATCC-CCL 2.2)	5-15-73	?	Clone of CCL 2	Negro	A	F	—	?	68	51-74	+	+	2*	+	—	
Detroit 6 (ATCC-CCL 3)	6-19-63	160	Sternal bone marrow (Patient with carcinoma of lung)	Caucasian	A	M	—	160	67	51-75	—	2*	3-4*	±	LI†	
Chang Liver (ATCC-CCL 13)	3-29-63	255	Normal liver	?	A	?	—	257	70	61-82	2*	+	4*	+	—	
KB (ATCC-CCL 17)	6-19-63	361	Epidermoid carcinoma of oral cavity	Caucasian	A	M	—	362	75	53-80	—	+	4*	+	LSC†	
AV <sub>3</sub> (ATCC-CCL 21)	9-17-63	270	Amnion	Negro	A	?	—	272	77	58-95	—	—	3*	+	LSC†	
FL (ATCC-CCL 62)	1-17-66	?	Amnion	?	A	?	—	?	71	68-142	+	+	3*	2*	LSC† 2 LI‡ MI§	
RPMI 2650 (ATCC-CCL 30)	11-25-66	22	Pleural effusion (Patient with carcinoma of nasal septum)	Caucasian	B	M	+	28	46	43-92	—	—	—	—	—	
RD (ATCC-CCL 136)	5-13-70	32	Rhabdo- myosarcoma	Caucasian	B	F	—	45	51	49-97	—	—	—	—	—	
Detroit 562 (ATCC-CCL 138)	11-24-69	43	Pleural effusion (Patient with carcinoma of pharynx)	Caucasian	B	F	—	44	64	58-128	—	—	—	—	LSC†	

\*No. of copies when more than one.

†Large submetacentric chromosomes (LSC).

‡Large isochromosomes (LI).

§Medium sized isochromosome (MI).

five more human cell lines have been examined and found to possess HeLa marker chromosomes. These cell lines are Minnesota EE, ATCC-CCL 4; L-132, ATCC-CCL 5; Detroit-98, ATCC-CCL 18; NCTC 2544, ATCC-CCL 19; and WISH, ATCC-CCL 25.

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## Vertical transmission of tumour resistance in guinea pigs

BOTH intact bacillus Calmette-Guerin (BCG)<sup>1-3</sup> and the methanol extraction residue (MER) fraction<sup>4</sup> of BCG have had extensive application in tumour immunotherapy. Treatment with MER nonspecifically stimulates both the cellular and humoral immune systems<sup>4,5</sup>, and has been successful in tumour immunotherapy in a number of animal models<sup>6,7</sup>. Experiments in our laboratories, using animals obtained from the Weizmann Institute, Rehovot, Israel, have shown that about 40% of strain 2 guinea pigs injected with MER or with the supernatant of sonically-disrupted BCG (BCG-SS), 1-2 months before intradermal inoculation with 10<sup>6</sup> cells of the transplantable line 10 hepatocarcinoma<sup>8,9</sup>, are resistant to the growth of this tumour<sup>10</sup>. Similarly, injection of MER into developing tumour nodules 7d after tumour cell implantation results in complete tumour regression in approximately 40% of the animals tested (M.A.W., P.M. and D.W.W., unpublished).

Previous studies have demonstrated that specific antibodies can traverse the placental barrier from maternal to foetal guinea pigs<sup>11</sup>. About 50% of the offspring of BCG-SS-treated female guinea pigs that became resistant to line 10 tumour were themselves resistant to challenges with line 10 tumour cells<sup>12</sup>.

We became interested in whether a similar transfer of protection against this tumour might also occur following treatment with MER, and what the mechanism of this vertical transmission might be. Line 10 hepatocarcinoma (B. Zbar, National Cancer Institute, Bethesda, Maryland) was maintained in ascites form by intraperitoneal passage in strain 2 guinea pigs (bred at Weizmann Institute). Tumour cells were collected and prepared for intradermal inoculation as described previously<sup>10</sup>. Nine tumour-free female guinea pigs, which had been treated with single doses of MER (0.5–1.0 mg) (Department of Drug Research and Development, National Cancer Institute) or BCG-SS (50 µg N) before or after tumour cell implantation and which were resistant to further challenge ( $10^7$  cells), were mated with normal males at least 60 d after their initial tumour cell challenge ( $10^6$  cells). The newborn guinea pigs of these matings were inoculated intradermally with  $10^6$  tumour cells when approximately 30 d old and numbers of survivors and mean days of death recorded (Table 1). Age-matched offspring of seven normal guinea pigs served as controls. Complete tumour regression was observed in 11 of the 26 experimental animals tested (42%), whereas a considerable delay in onset of death was observed in nine others (experiments 2, 5, 6 and 7). No differences were observed between the capacities of male and female newborns to survive tumour challenge. Growing tumour

nodules were observed in all guinea pigs by 4 d after tumour cell implantation. In those animals which developed complete resistance to the tumour, these nodules became progressively smaller from about 7 d after tumour challenge and eventually disappeared. In the case of many guinea pigs which eventually succumbed, tumour growth was markedly retarded at the site of implantation, and it was only following the appearance of palpable metastatic lesions in the regional lymph nodes that progressive tumour enlargement and death ensued. In contrast, rapid tumour growth at the site of initial challenge followed by death was observed with each of 19 age-matched controls ( $P < 0.001$ ;  $\chi^2$  test). In three cases (Table 1, experiments 8–10), the tumour resistant female offspring of MER-treated animals were themselves mated with normal males and the third generation guinea pigs challenged with  $10^6$  tumour cells as described above. Vertical transmission of tumour resistance was again observed; three of the eight third generation offspring survived tumour challenge as opposed to none of the six age-matched controls.

To evaluate whether a transplacental transfer of immune factors was taking place, some of the maternal tumour-resistant animals of Table 1 and their offspring were skin tested, 3 d before and 3 weeks after tumour cell challenge, with 0.1 ml each of purified protein derivative of *Mycobacterium* (PPD, Ministry of

Table 1 Vertical transmission of tumour resistance

Experiment	History of mother	Age of newborns at challenge (d)	Number in litter	Number of survivors	Mean day of death of non-survivors
1	BCG-SS immunoprophylaxis cure	30	2	2	—
	Controls	30	2	0	54
2					
Litter a	MER immunoprophylaxis cure*	30	2	0	71
Litter b	MER immunoprophylaxis cure	32	1	1	—
	Controls	28	2	0	54
3	MER immunoprophylaxis cure*	29	3	0	47
	Controls	30	2	0	52
4					
Litter a	MER immunotherapy cure	31	4	1	40
Litter b	MER immunotherapy cure	28	1	1	—
	Controls	29	2	0	55
5	MER immunotherapy cure	25	4	1	83
	Controls	27	4	0	55
6					
Litter a	MER immunotherapy cure	29	3	2	81
Litter b	MER immunotherapy cure	31	2	1	79
	Controls	30	4	0	61
7	MER immunotherapy cure	28	4	2	78
	Controls	28	3	0	58
8	Female survivor of experiment 1	28	3	0	69
	Controls	32	2	0	62
9	Female survivor of experiment 2, litter b	31	2	1	64
	Controls	29	2	0	58
10	Female survivor of experiment 4, litter b	30	3	2	64
	Controls	30	2	0	60
	Total number of experimental animals		34	14	65
	Total number controls†		25	0	—

\*Same mother; litters born 4 months apart.

†Significance of comparison: experimentals-controls,  $P < 0.001$ .

**Table 2** Effect of cross rearing of newborn guinea pigs on tumour survival

Offspring of	Nursed by	Number in litter	Number of survivors	Mean day of death of non-survivors
Tumour-resistant mother 1	Normal mother 1	2	2	—
Tumour-resistant mother 2	Normal mother 2	4	2	61
Tumour-resistant mother 3	Normal mother 3	3	0	65
Normal mother 1	Tumour-resistant mother 1	2	0	63
Normal mother 2	Tumour-resistant mother 2	2	0	59
Normal mother 3	Tumour-resistant mother 3	4	0	66
Tumour-resistant male 1	Normal mother 4	4	0	63
Tumour-resistant male 2	Normal mother 5	3	0	68
Tumour-resistant male 3	Normal mother 6	3	0	59
Tumour-resistant male 4	Normal mother 7	4	0	62

Agriculture, Weybridge, UK), soluble tumour antigen extract (SA-10, prepared by 3 M KCl extraction as described previously<sup>13</sup>), soluble normal liver antigen extract (SA-N, prepared as above<sup>13</sup>), and isotonic saline (NaCl). Areas of induration were measured after 24 and 48 h. Tumour-resistant mothers showed significant delayed cutaneous hypersensitivity (DCH) to both PPD and SA-10 but not to either SA-N or NaCl. There was no significant response by either tumour-resistant or susceptible offspring to these antigens before challenge with tumour cells. Three weeks after challenge, however, all the offspring of tumour-resistant and control mothers had DCH reactions to SA-10. Thus, DCH to SA-10 and PPD did not seem to be transmitted transplacentally to these newborn guinea pigs, and the development of DCH to SA-10 after tumour challenge was not related to their ultimate survival.

The possibility that vertical passage of immune serum elements might occur was assessed. Each of three MER-treated and tumour-resistant guinea pigs were injected intramuscularly with  $10^7$  tumour cells to stimulate both cellular and humoral anti-tumour immunity. These animals, all of which showed strong DCH reactivity to SA-10 (10 µg), were bled 3 and 7 days after immunisation, and 1 ml aliquots each of serum, plasma or whole blood cells were passively transferred by cardiac puncture to ten normal 25-d-old recipients. The latter were challenged 7 d later with  $10^6$  tumour cells. No retardation of either tumour growth or onset of death was observed in any of these animals.

To study the possible role of the milk of nursing mothers as a source of tumour resistance, the newborn offspring of each of three MER-immunotherapy-cured females and three female controls were interchanged as indicated in Table 2. The 17 newborn guinea pigs in this 'criss-cross' experiment were challenged with  $10^6$  cells when about 30 d old. Four of the nine offspring of MER-treated, tumour-resistant mothers were themselves resistant to growth of the line 10 hepatocarcinoma, in spite of having been suckled on normal lactating females. By contrast, tumour-resistant females seemed to be unable to transfer resistance by nursing to any of the eight offspring of control mothers. This finding is in agreement with the observation that guinea pig IgG antibodies are not transmitted through the milk<sup>14</sup>. Parallel experiments (Table 2) showed that none of the 14 newborn guinea pigs born to normal mothers but fathered by MER-treated tumour-resistant males resisted challenge with line 10 cells.

In summary, vertical transmission of resistance to the transplantable guinea pig line 10 hepatocarcinoma from MER-treated tumour-resistant mothers has been demonstrated. The nature of the mechanisms responsible for this protection remains to be elucidated. It is clear, however, that they are not related to factors transmitted through the milk. The fact that a significant proportion of the third generation offspring of resistant guinea pigs are also tumour-resistant makes it unlikely that this phenomenon is attributable to any lingering effect of MER or BCG-SS. Tests for DCH did not reveal evidence of cellular anti-tumour immune activity in the offspring of tumour-immune mothers, before skin challenge with line 10 hepatocarcinoma cells. Nevertheless, the considerations that small quantities of anti-tumour antibodies and/or specifically sensitised small lymphocytes may be transported across the placental barrier cannot be ruled out and are presently under investigation.

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## Gene that controls initiation of chromosome replication and prophage induction in *Bacillus subtilis*

THE mechanism of initiation of the chromosome replication cycle in bacteria is beginning to be elucidated by genetic and biochemical studies<sup>1-3</sup>. But little is known about what controls the initiation frequency which has a key role in regulating the rate of DNA synthesis in response to the change in bacterial growth rate<sup>4,5</sup>. The existence of such a regulatory entity—initiator—synthesised in harmony with the increase in cell mass or the change in the cell or replication cycle, has been hypothesised<sup>6,7</sup>. We have investigated the nature of the hypothetical regulator, based on the assumption that the induction of prophage and the initiation of host cell chromosome replication would be controlled by a common regulator directly or indirectly. The assumption is based on observations that the inducibility of prophages and the initiation frequency of chromosome replication share common features in their responses to the bacterial growth rate and to the inhibition of DNA replication<sup>4,5,8-10</sup>. We assumed that the regulatory material, substance X, accumulates in the cell depending on growth rates and is consumed to initiate each replication cycle, and

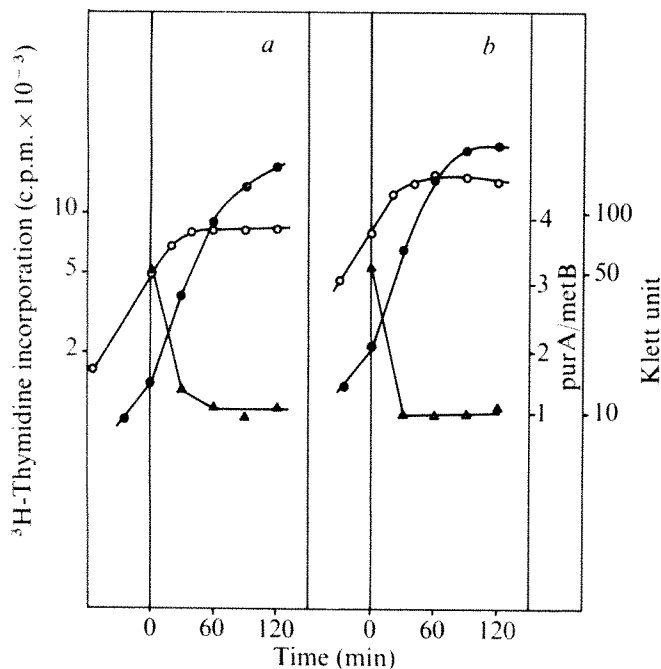


that an unbalanced accumulation of X caused by the inhibition of DNA synthesis would provoke prophage induction. If this assumption is correct it should be possible to isolate conditional mutants in which both the initiation of chromosome replication and prophage induction are prevented in non-permissive conditions.

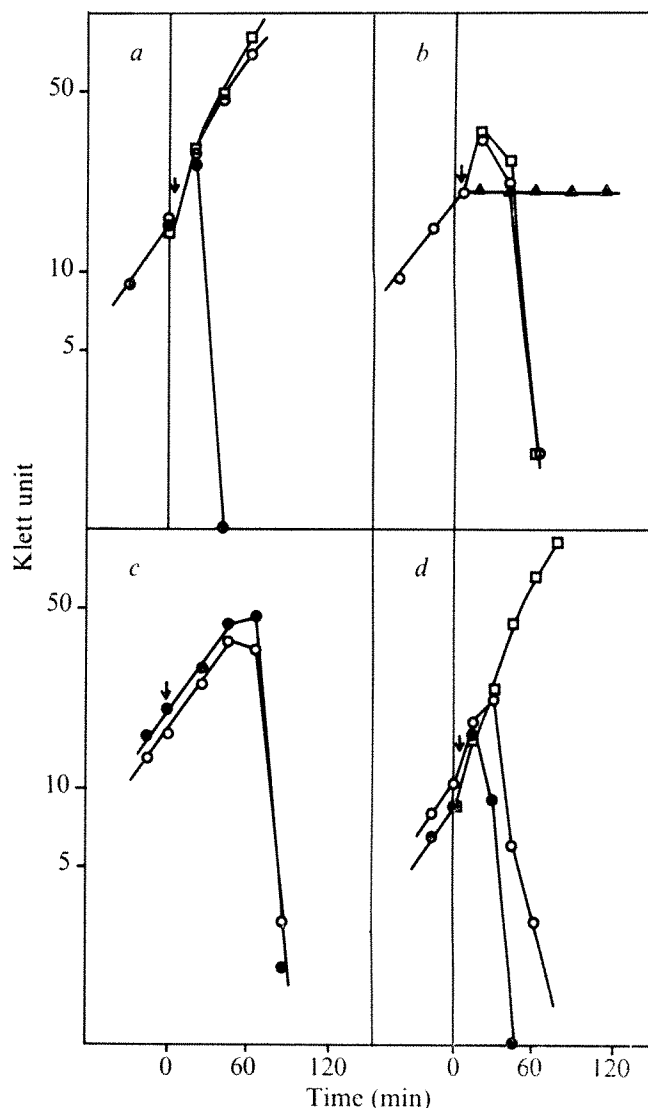
Induction of a temperate phage SPO2 in *Bacillus subtilis* by 6-*p*-hydroxyphenylazouracil (HPUra)<sup>11</sup> was used as a test system because the uracil analogue inhibited semi-conservative DNA replication by interacting specifically with DNA polymerase III of *B. subtilis*, without affecting either the repair synthesis<sup>12</sup> of the cell or the replication of the phage DNA. Most of the commonly used inducing agents, such as ultraviolet irradiation, mitomycin C (mit C) and nalidixic acid are not suitable for this test because they cause damage in the DNA molecule, which in turn may affect prophage inducibility<sup>13,14</sup>.

*B. subtilis* 168 (trp<sup>-</sup>), CRK1000, was lysogenised with the phage SPO2 and mutated with nitrosoguanidine<sup>10</sup>. The resultant mutagenised lysogens were grown in a rich Lp medium<sup>15</sup> at 30 °C and then heated to 45 °C with the addition of HPUra 5 min after heating. After 60 min of incubation at 45 °C survivals were collected on a filter paper and washed to remove HPUra. From these cells temperature-sensitive (*ts*) mutants were isolated by a conventional replica method. Properties of 133 *ts* mutants isolated in several experiments are summarised in Table 1. Thirty-seven mutant lysogens which showed both temperature-sensitive growth and temperature-sensitive induction (no induction by HPUra at 45 °C were isolated either spontaneously or after treatment with nitrosoguanidine. A double selection by repeated HPUra induction at 47 °C

isolated this type of mutant. The mutation in the host chromosome was demonstrated directly by introducing the *ts* character into a recipient, thy<sup>-</sup> cell. The resultant transformants carried *ts* and thy<sup>-</sup> characters and therefore DNA synthesis in them was measured by <sup>3</sup>H-thymidine incorporation. Ten out of 31 transformed mutant strains



**Fig. 1** Growth and DNA synthesis of *dna-ts* initiation mutant, CRK2605 (*ts*1315) and CRK2001 (*ts*27). Cells of CRK2605 or CRK2001 were grown in Lp medium containing necessary amino acid and <sup>3</sup>H-thymidine (0.5  $\mu$ Ci per 5  $\mu$ g per ml) until an early exponential phase when the temperature of the culture was shifted quickly to 47 °C. At various times before and after the shift, 0.5 ml samples were taken to measure radioactivities incorporated into the DNA fraction after incubation in 0.2 M NaOH at 80 °C for 30 min, followed by precipitation in 10% trichloroacetic acid. In parallel with this measurement DNA was isolated from 5-ml samples and its transforming activities for adenine and methionine were determined. Marker frequency analysis was done as before<sup>18</sup>. A mutant strain CRK3000 (*leu*<sup>-</sup>, *metB*<sup>-</sup>, *purA*<sup>-</sup>, *hisA*<sup>-</sup>) was used as recipient. *a*, CRK2605; *b*, CRK2001.  $\circ$ , <sup>3</sup>H-thymidine incorporation;  $\bullet$ , cell turbidity in Klett unit;  $\blacktriangle$ , *purA/metB*.



**Fig. 2** Effects of HPUra and mitC on induction and infection of SPO2 in a mutant carrying *ts-dna-ind* mutation, CRK2605. CRK2605 or its lysogen, CRK2605(SPO2), was grown in complete Lps medium (which is Lp plus MgSO<sub>4</sub>, 5 mM, and MnCl<sub>2</sub>, 0.5 mM) at 30 °C. At an early exponential phase, cells were treated as below and the turbidity was measured using a Klett colorimeter. After the last turbidity measurement, all the culture was added to Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (final 25 mM) and stored at 4 °C overnight to determine the phage titre by the method of Okubo and Romig<sup>19</sup>. *a*, CRK2605(SPO2) was heated to 47 °C at time zero and drugs were added 5 min later. Incubation was continued at 47 °C.  $\square$ , No drug,  $4.2 \times 10^6$  plaque-forming units per ml (PFU ml<sup>-1</sup>) in the lysate;  $\circ$ , with HPUra (70  $\mu$ M),  $2.5 \times 10^7$  PFU ml<sup>-1</sup>;  $\bullet$ , with mit C (1  $\mu$ g ml<sup>-1</sup>),  $6.3 \times 10^6$  PFU ml<sup>-1</sup>. *b*, CRK2605 was heated to 47 °C at time zero and the phage SPO2 (multiplicity of infection 0.6) was added to the culture 5 min after heating, with or without drug. Incubation was continued at 47 °C.  $\square$ , No drug added,  $2.4 \times 10^6$  in the lysate;  $\circ$ , with HPUra,  $2.1 \times 10^6$  PFU ml<sup>-1</sup>;  $\blacktriangle$ , with HPUra and rifampicin (2  $\mu$ g ml<sup>-1</sup>),  $7 \times 10^5$  PFU ml<sup>-1</sup>. *c*, CRK2605(SPO2) was added with drugs at time zero and incubation continued at 30 °C.  $\circ$ , With HPUra,  $1.9 \times 10^6$  PFU ml<sup>-1</sup> in the lysate;  $\bullet$ , with mit C,  $1.7 \times 10^6$  PFU ml<sup>-1</sup>. *d*, For comparison, the mutant lysogen carrying *ts*27, CRK2001(SPO2), was grown at 30 °C, heated to 47 °C and added with drugs as in (*a*).  $\square$ , No drug addition,  $1.0 \times 10^6$  PFU ml<sup>-1</sup> in the lysate;  $\circ$ , with HPUra,  $4.7 \times 10^6$  PFU ml<sup>-1</sup>;  $\bullet$ , with mit C,  $3.9 \times 10^6$  PFU ml<sup>-1</sup>.

**Table 1** Isolation of *ts* mutants of *B. subtilis* from surviving SPO2 lysogens after inducing treatments at high temperature

Mutagenised by NTG	Selection methods	Lysogenic cells ( <i>trp</i> <sup>-</sup> (SPO2))		Transformants ( <i>thy</i> <sup>-</sup> , <i>ts</i> )		Total
		<i>ts</i> mutants	HPUra-induction <i>ts</i> mutants	<i>dna-ts</i> mutants Initiation	Others* Elongation	
+	—	23	3	0	3	3
+	HPUra	74	24	9	2	21
+	HPUra-ultraviolet	25	4	0	0	1
—	HPUra-HPUra	11	6	1	0	6
Total		133	37	10	2	31

The SPO2 lysogen of *B. subtilis* 168 (*trp*<sup>-</sup>), CRK1000 (SPO2), was grown in Lp medium<sup>18</sup>, and treated with nitrosoguanidine (NTG) at 30 °C as before<sup>10</sup>. NTG-treated cells were washed and grown at 30 °C in minimal medium Cg (ref. 13) to minimise prophage induction after NTG treatment. After about 60 min cells began to increase when they were mixed with 20% glycerol and frozen in liquid nitrogen. Mutagenised cells were thawed immediately and grown in Lp medium at 30 °C for one generation. One sample (column 1) was plated directly on BHI agar<sup>10</sup> without further treatment. Other cells were heated to 45 °C, added to HPUra (70 µM) after 5 min and incubated for 60 min at 45 °C. Cells lysed at about 45 min. Surviving cells were collected by filtration, washed and then plated on BHI agar (column 2). In two samples (columns 3 and 4), survivals from the first HPUra treatment were grown in Lp medium at 30 °C and then heated to 45 °C. After 5 min at 45 °C cells were either added with HPUra (70 µM) or irradiated with ultraviolet light at 4 µW cm<sup>-2</sup> for 2 min. Cells were further incubated at 45 °C for 60 min and cells remaining after lysis were filtered, washed and plated on BHI agar. For the sample in the last column cells grown in Lp medium at 30 °C were used without NTG treatment. Cells plated on BHI agar plate after these various selections were grown at 30 °C and the colonies appearing after 12 h were replica plated on BHI and incubated at 45 °C. Colonies that failed to grow at 45 °C were isolated as *ts* mutants. *ts* lysogens thus obtained were grown in Lp medium at 30 °C and HPUra (70 µM) was added at an early exponential phase either at 30 °C or 5 min after heating to 45 °C. Cell lysis and phage production (detected by a spot test) were followed at both temperatures. The number of *ts* mutants that lysed and produced phage at 30 °C and did not lyse and produced few phages at 45 °C are listed. DNA was isolated from the HPUra-induced *ts* mutants (total 37) as described before<sup>18</sup>. Competent cells of CRK1500, *thy*<sup>-</sup>, *leu*<sup>-</sup>, were transformed with these DNA samples of saturated concentration<sup>18</sup>. From *leu*<sup>+</sup> transformants, *ts* transformants were selected and immunity to and infectivity by SPO2 were examined to confirm that the SPO2 genome was not co-transformed with *ts* markers. Of the 37 mutants, 31 were transformed successfully. The transformants (*ts*, *thy*<sup>-</sup>) were grown at 30 °C in the Lp medium containing <sup>3</sup>H-thymidine (0.5 µCi per 5 µg per ml) and DNA synthesis and growth were determined by <sup>3</sup>H-thymidine incorporation and turbidity measurement respectively, after heating the exponentially growing cell to 47 °C. In initiation mutants, the residual amount of DNA synthesis was 50–100%, equivalent to the DNA present at the time of heating. The same amount was less than 20–30% in the elongation mutants.

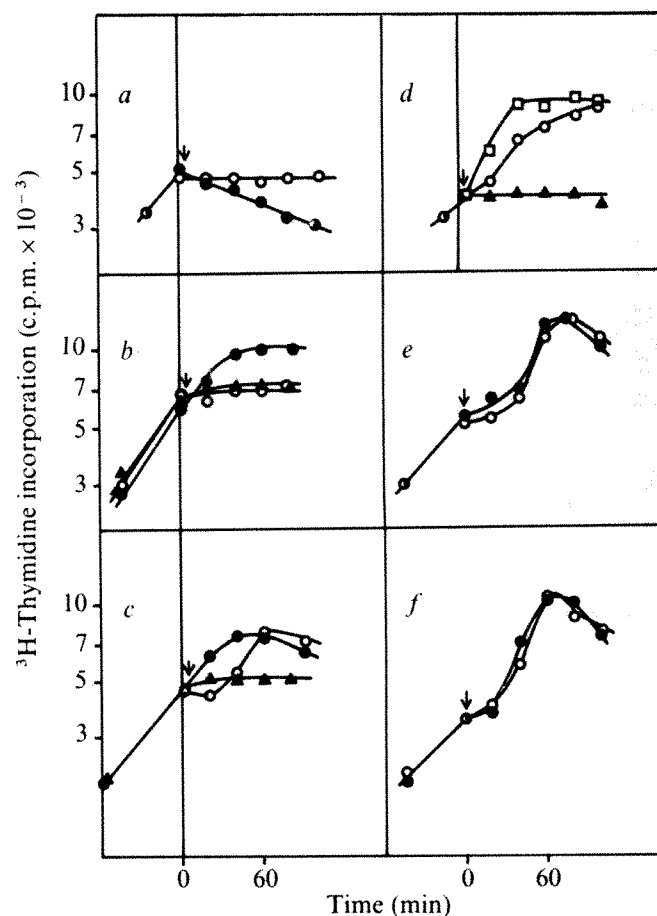
\* These include the mutants in which more than 100% of DNA was synthesised at 47 °C and those in which both DNA synthesis and growth were inhibited at 47 °C.

were found to be *dna-ts* initiation mutants. Marker frequency analysis during the incubation of these mutants at 47 °C clearly showed that the initiation of new rounds of replication was prevented immediately after heating (Fig. 1).

As expected, when these mutants were lysogenised with a wild-type SPO2 the prophage was induced by HPUra at 30 °C but not at 47 °C, as shown by cell lysis (Fig. 2), the phage titre liberated into the medium (legend to Fig. 2) and the incorporation of <sup>3</sup>H-thymidine in the presence of HPUra, which is a direct indication of the replication of the SPO2 DNA (Fig. 3). Data in Figs 2 and 3 show that (1) the sensitivity of chromosome replication to HPUra was not altered by the mutation, (2) the expression of early genes and the autonomous replication and maturation of SPO2 occurred normally at 47 °C in the presence of HPUra, as indicated by a successful infection by SPO2 and induction by mit C at 47 °C. Therefore we conclude that the change in the prophage from a repressed to a derepressed state caused by the cessation of chromosome replication by HPUra is prevented specifically at the non-permissive temperature.

From this evidence we conclude that the mutation in this group of *ts* mutants affects, at the non-permissive temperature, both the initiation of chromosome replication and the prophage induction by HPUra. We call them collectively *ts-dna-ind* mutants. No *ts-dna-ind* mutant was isolated when ultraviolet treatment was used at the second selection cycle in place of HPUra, and mit C

caused SPO2 induction in the mutants at 47 °C even in the presence of HPUra, indicating that ultraviolet light and mit C caused induction through a mechanism at least partly different from that of HPUra. These mutants carried a single mutation and the induction by HPUra occurred at 47 °C in *ts*<sup>+</sup> revertants. Furthermore, both initiation of chromosome replication and the induction of SPO2 by



**Fig. 3** Induction and infection of the phage SPO2 in *dna-ts* initiation mutants assayed by <sup>3</sup>H-thymidine incorporation in the presence of HPUra. Two *dna-ts* initiation mutants, CRK2605 and CRK2001, and their SPO2 lysogens were grown in complete Lps medium containing <sup>3</sup>H-thymidine (0.5 µCi per 5 µg per ml) at 30 °C. At an early exponential phase, cells were treated as shown below and DNA synthesis was measured as described in the legend to Fig. 1. □, Without drug; ○, with HPUra; ●, with mit C and HPUra; ▲, with HPUra and rifampicin. *a*, CRK2605; *b*, CRK2605(SPO2); *c*, CRK2001(SPO2); *d*, CRK2605. The cell was cultured at 30 °C and then the temperature of the culture was shifted quickly to 47 °C. After 5 min cells were added to the drugs and in (*d*) infected with the phage SPO2 (multiplicity of infection 0.6) simultaneously; *e*, CRK2605(SPO2) was cultured at 30 °C; *f*, CRK2001(SPO2) was cultured at 30 °C.

HPUra occurred when mutant cells (non-lysogen and SPO2 lysogen, respectively) were treated at 47 °C for 60 min and then cooled at 30 °C. Chromosome replication was re-initiated even in the presence of chloramphenicol, suggesting that the *ts* component was readily reactivated at 30 °C (data not shown).

Genetically it is interesting to ask whether these *dna-ind* mutations are clustered in one gene or dispersed in many genes. Recombination tests using two mutants carrying the *dna-ind* mutations as recipients revealed that the 10 *ts-dna-ind* mutations were not identical but closely linked, having recombination indices of 0–0.3, suggesting that they are located within one gene<sup>16</sup>. Furthermore, these mutations were tightly linked to *dna-ts27*, a mutation carried by a *ts-dna* initiation mutant isolated in our laboratory<sup>17</sup>. Phenotypes of the mutant with respect to the *ts* character of the initiation of the chromosome replication were indistinguishable from *dna-ind* mutations (Fig. 1). SPO2, however, was induced by HPUra in the *ts27* mutant even at 47 °C (Figs 2 and 3). These results suggest that the initiation of chromosome replication and prophage induction are affected independently by the *dna-ind* gene product. A single mutation in this gene may alter either one of the two functions or both functions simultaneously.

We assume that X has dual functions in the initiation of chromosome replication and prophage induction. This is suggested by the general resemblance between the two events in terms of their responses to bacterial growth rates. The highly selective concentration of the specific type of the *dna-ts* initiation mutant by the selection method used in this experiment revealed close relationship between the two events. Furthermore, the finding of the *dna-ind* gene and pleiotropic effects of its mutations support the existence of the suggested substance X. The relationship between the product of this gene and X is unclear at present but the simplest interpretation is that the product is X itself.

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## Differentiation of B and T mouse lymphocytes in cell suspension and smears

THE differentiation of B and T lymphocytes by scanning electron microscopy and, in live suspensions, by light microscopy has led to controversy about their different surface characteristics<sup>1,2</sup>. They can be demonstrated serologically by the presence or absence of specific antigen markers in various animal species, including man. B cells can be re-

cognised among mouse lymphocytes by surface immunoglobulin staining under fluorescent microscopy<sup>4</sup>. The surface morphology of lymphocytes has been distinguished by light microscopy using Nomarski differential interference<sup>3</sup>. We have now used the Hoffman modulation contrast microscopy system<sup>6,7</sup> and confirmed that B and T lymphocytes have different surface characteristics. This technique makes it possible to observe both live cells and air-dried smears, and we have demonstrated the feasibility of restoring the distinct surface characteristics in blood smears.

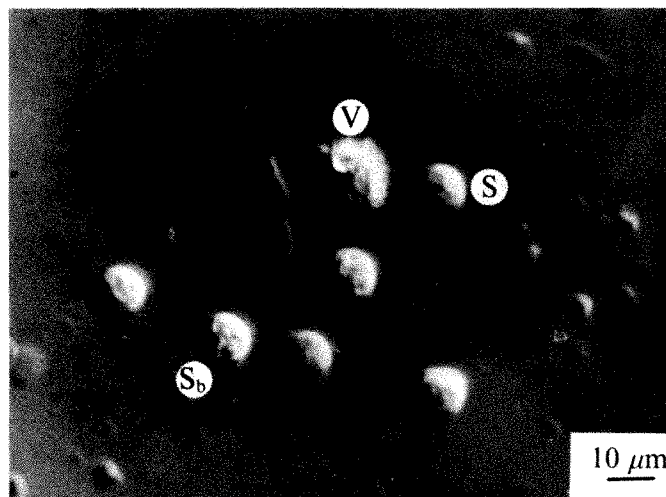
C57BL/6, BALB/c and Dublin (ICR) Swiss mice, 4–8 weeks old, and of both sexes, were used. Peripheral blood was collected from the orbital sinus in 24 heparinised haematocrit tubes, and pooled in a syringe containing heparin (50 U). Pooled blood was layered on a Ficoll-Hypaque mixture for density gradient separation of lymphocytes and platelets by Boyum's method<sup>4</sup>. This layer was then removed and centrifuged at 400g and resuspended in Eagle's minimum essential medium (MEM) with or without 5% foetal calf serum (FCS).

Spleen and thymus from individual mice were pressed separately and gently through a stainless steel wire mesh in 5 ml of MEM with or without 5% FCS, followed by passage through a 26-gauge needle.

Fluorescein-conjugated anti-mouse IgM was obtained from Meloy Laboratories. Lymphocyte pellets obtained from a single spleen and thymus, or density-gradient-separated peripheral blood lymphocytes, were sedimented at 400g for 5 min and resuspended in 0.2 ml of MEM, then combined with 0.2 ml of the fluorescein-conjugated anti-serum at a protein concentration of 0.8 mg ml<sup>-1</sup>. The reaction mixture was incubated at 37 °C for 30 min with occasional shaking. Cells were washed three times in MEM only and resuspended in 1 ml of MEM containing FCS and applied to a microscope slide chamber made of two No. 1 glass coverslips mounted on the slide with Corning silicone vacuum grease. These supported a third coverslip, No. 1 1/2 thickness, making a thin chamber closed on two sides<sup>9</sup>. The chamber was filled by applying the cell suspension to one of the open sides, and inverted for 1–5 min to allow the cells to settle on the coverslip. When the chamber was reversed, many cells were hanging from the underside of the coverslip. Before examination, all cell suspensions were tested for cell viability by Trypan blue exclusion and found to contain more than 90% viable cells.

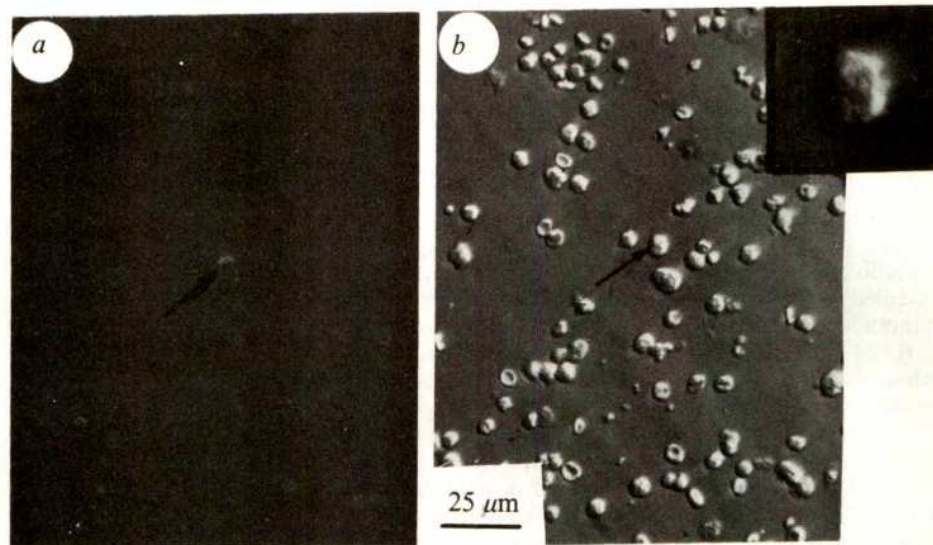
Peripheral blood obtained for the cell suspensions was also used for blood smears. Duplicate blood smears were

**Fig. 1** Thymus suspension from Dublin Swiss mouse showing the three types of surface characteristics of lymphocytes: villous (V), smooth (S), smooth, with a broad base surface difference (S<sub>b</sub>) (×40 achromat objective and ×10 ocular, Kodak 35-mm film No. 5032).





**Fig. 2** *a*, Spleen suspension from Dublin Swiss mouse tagged with fluorescein isothiocyanate anti-mouse IgM; crescent-shaped fluorescence from a villous lymphocyte, *b*, modulation contrast of same field. The arrow points to the same cell ( $\times 40$  achromat objective  $\times 3.3$  ocular, Polaroid film Type 107 and Kodak SO 410 35-mm film (inset)).



prepared from individual mice, one acted as a control slide stained with Wright's stain for differential white blood cell determination, the other for Hoffman modulation contrast visualisation. The unstained smear was processed within 24 h by immersion in physiological buffered saline at 20 °C for 30 s for red cells to lysis. A coverslip was then applied to the wet surface, excess fluid removed and the edges sealed with nail polish. Differential counts were made for lymphocytes, granulocytes and monocytes.

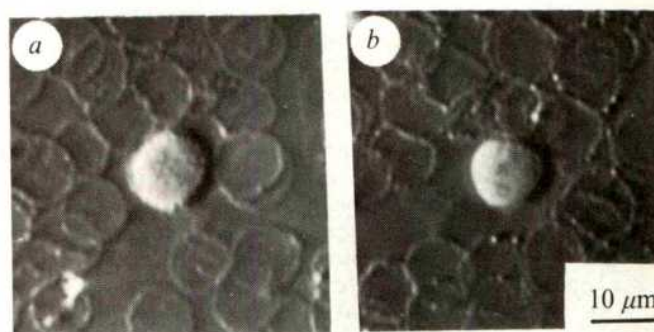
The Hoffman modulation contrast system was adapted to an ordinary Nikon bright-field transmission microscope, and to the Olympus reflected light fluorescent system on the Vanox transmission microscope by Modulation Optics Inc. The system involves a modulator placed off-centre in the back focal plane of the objective and an off-centre slit beneath the condenser. This creates intensity contrast of phase gradients, producing a three-dimensional image. The objective with the modulator is used with both reflected fluorescent and transmitted modulation contrast without degradation of the fluorescent image.

Suspensions of live lymphocytes from the peripheral blood, spleen and thymus of mice, examined with a  $\times 40$  achromat, objective, revealed two types of lymphocyte, some with fine, villous projections and others which were smaller, but with smooth surfaces (Fig. 1). There were also cells with essentially smooth surfaces, but which contained broad based projections (Fig. 1). When these suspensions were examined in the enclosed slide chamber for up to 1 h there was no essential change in their surface characteristics. Most lymphocytes in the lymphoid organ suspensions examined had smooth surfaces. Lymphocytes with villous

surfaces were fewer and varied in quantity with 0.2–2% in the thymus, an average of 13% in the peripheral blood and 23.2% in the spleen (Table 1).

Surface morphology of lymphocyte suspensions from the thymus incubated with fluorescein-conjugated anti-mouse IgM revealed less than 1% positive cells. When transmitted modulation contrast microscopy was directed to the same field of view, all fluorescent lymphocytes had villous surfaces. Less than 1% were villous and negative and all smooth lymphocytes were negative (Table 1).

Fluorescent cells revealed typical staining, with Ig limited to the surface in the form of a crescent or complete peripheral rim, or a stippled area. Specimens preincubated with unlabelled anti-mouse gamma globulin did not fluoresce when incubated with fluorescein-conjugated anti-IgM reagent. Suspensions of spleen and peripheral blood lymphocytes, which were observed but not counted, revealed similar patterns of fluorescence, although some villous lymphocytes did not fluoresce (Fig. 2).



**Fig. 3** Peripheral blood smear from C57BL/6 mouse, air-dried and restored. *a*, B lymphocyte with restored surface projections and serrated border; note background of lysed red blood cells. *b*, Smooth surface T lymphocyte with smooth border ( $\times 40$  achromat objective,  $\times 10$  ocular, Kodak SO 410 35-mm film).

**Table 1** Quantitation of lymphocytes with stated appearances as viewed by Hoffman modulation contrast microscopy

Organ cell suspension	Mouse	Smooth cells	Villous cells	% Villous cells
Peripheral blood	BALB/c	431	63	13
	Dublin Swiss	348	52	av. 13 (10–17)
Spleen	C57BL/6	367	133	av. 26.6 (25–30)
	BALB/c	156	44	av. 22 (21–23)
	Dublin Swiss	860	240	av. 22 (18–31)
Thymus	C57BL/6	998	2	0.2
	BALB/c	497	3	0.6
	Dublin Swiss	294	6	2.0
	Dublin Swiss	550*	1* + 3†	0.5

\*Non-fluorescent. †Fluorescent cells counted by reflected fluorescent and transmitted modulation contrast microscopy.

The characteristic leukocytes found in mouse blood smears were identified by modulation contrast as granulocytes, monocytes and lymphocytes. There was no appreciable loss of adherent leukocytes after a 30-s immersion in physiological buffered saline. Differential counts with standard Wright's stained slides revealed comparable differential counts with duplicate specimens differentiated for surface characteristics by modulation contrast microscopy (Table 2). The lymphocyte population included cells with small surface projections, closely grouped and extending from the peripheral borders, giving a serrated appearance (Fig. 3a), which were designated B cells. Other lymphocytes had smooth peri-



pheral borders and smooth surfaces, including some with one or two flattened projections or folds (Fig. 3b) and they were designated T lymphocytes. Counts of normal lymphocytes in each smear specimen gave a ratio of 89–92% of T cells and 8–11% of B cells (Table 2). The ratio of villous and smooth surface lymphocytes in the peripheral blood suspensions (average values of 13% and 87%) was similar with that of the restored blood smear preparations. This differentiation of T and B cells supports the observations of Rabellino *et al.*<sup>4</sup>. Similar surface differences have been revealed by Nomarski interference contrast on unfixed human<sup>10</sup> and guinea pig<sup>3</sup> lymphocyte suspensions.

We cannot reconcile our observations with those of Alexander and Wetzel<sup>2</sup>, who found no differentiation of villous and smooth cells among mouse and human lymphocyte populations prepared for scanning electron microscopy. They showed that the smooth lymphocyte population observed by scanning electron microscopy by Polliak *et al.*<sup>1</sup> may have resulted because fixation smoothed the surface structures nonspecifically. Our observations on living cell suspensions indicate no surface changes on smooth or villous surfaces during up to 1 h.

**Table 2** Comparison of leukocyte count in Wright-stained and modulation contrast microscopy of blood smears of mice

Mouse	Wright stain			Modulation contrast					
	L	G	M	T	B	%T	%B	G	M
No. 1-BALB/c	85	13	2	80	9	90	10	10	1
No. 2-BALB/c	82	16	2	78	6	92	8	14	2
No. 3-BALB/c	89	10	2	81	8	91	9	10	1
No. 1-C57BL/6	87	10	3	77	9	90	10	12	2
No. 2-C57BL/6	88	11	1	78	9	89	11	10	3
No. 3-C57BL/6	88	10	2	78	10	89	11	10	2

G, Granulocytes; M, monocytes; T, T lymphocytes; B, B lymphocytes.

In all cases the anti-mouse IgM fluorescent stain selected cells with villous projections, whereas if the environment changed the villous cells to smooth cells, the stain would have been expected among the smooth population. Some villous lymphocytes did not fluoresce, perhaps because the IgM antiserum was not specific for those surfaces: another heavy chain gamma globulin fraction of antiserum could be reactive.

Although with restored preparations it was possible to distinguish B and T lymphocytes, villous projections were shorter and less distinct. Conditions for viewing smears are optimal when preparations are restored within 24 h at room temperature. Optimal physical conditions for maintaining dried smears have not been explored fully.

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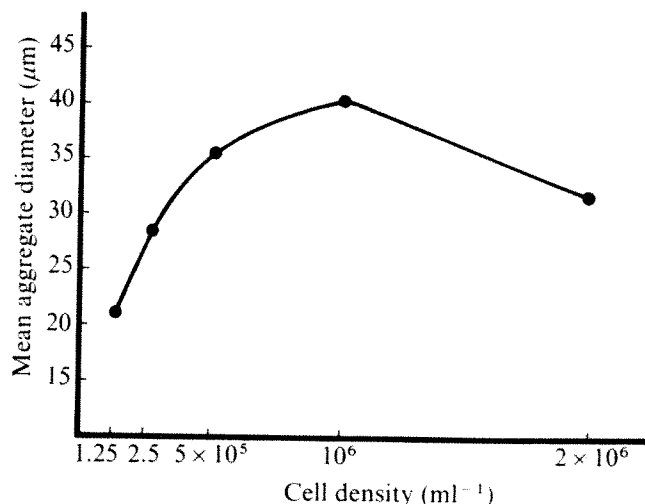
## Inhibitory effect of dibutyl cyclic AMP and theophylline on the aggregation of human breast tumour cell line BT-20

DISSOCIATED cells from embryos are able to reaggregate to form multicellular structures resembling those of the original tissue<sup>1,2</sup>, this ability being inversely proportional to the age of the embryo<sup>1,3</sup> or to the age of the cells in tissue culture<sup>4</sup>. This intracellular adhesion is tissue specific, and is believed to have a key role in growth and morphological differentiation<sup>5</sup>. It has also been noted that many tumour cell lines can reaggregate in the same condition as embryonic cells<sup>6–10</sup>. Leighton has observed the formation of small aggregates when tumour cells were grown in cellulose sponge<sup>11</sup>. He directs attention to the fact that many clinical carcinomas appear as aggregates or nests of cells, and suggests that factors intrinsic to the formation of aggregates are involved in the spread of cancer.

It has been shown that cyclic AMP may influence factors of the cellular membrane such as concanavalin A (con A) agglutination<sup>12</sup>, surface antigens on cells<sup>13</sup>, morphology of cells<sup>14</sup>, contact inhibition of growth<sup>15</sup>, and adhesion of cells to glass<sup>16,17</sup>. We report here that dibutyl (db) cyclic AMP and theophylline inhibit the aggregation of human mammary tumour cell line BT-20 (ref. 18).

The method of aggregating the cells is essentially that of Moscona<sup>1</sup>. BT-20 cells were grown in the medium RPMI 1640, with 10% foetal calf serum and 20  $\mu\text{g ml}^{-1}$  of insulin added. Suspensions of single cells were prepared from confluent cultures by trypsin and EDTA. Aliquots of cell suspensions (3 ml) containing  $10^4$ – $2 \times 10^6$  cells  $\text{ml}^{-1}$  were placed in 25-ml Erlenmeyer flasks and shaken on a gyrotary shaker for 24 h at 37 °C at 72 r.p.m., with a radius of rotation of 1.25 cm. The cells were washed twice in serum-free medium and then suspended in RPMI 1640 buffered with 20 mM HEPES to give the desired cell density. Serum and insulin were omitted from the medium during experiments, except where the effect of serum was studied. Stock solutions of db cyclic AMP (10 mM), cyclic AMP (10 mM), theophylline

**Fig. 1** Effect of cell density on aggregation size of BT-20 cells. Single cell suspensions at  $1.25 \times 10^4$  to  $2 \times 10^6$  cells  $\text{ml}^{-1}$  in RPMI medium without serum were placed in 25-ml Erlenmeyer flasks in 3-ml aliquots. The cell suspensions were incubated in a gyrotary shaker at 72 r.p.m. for 24 h. Samples (0.5 ml) were removed from each flask and placed in a plastic Petri dish. Photomicrographs of the aggregates were taken and the diameter of the aggregates measured. The mean size of aggregates from four flasks is shown here.



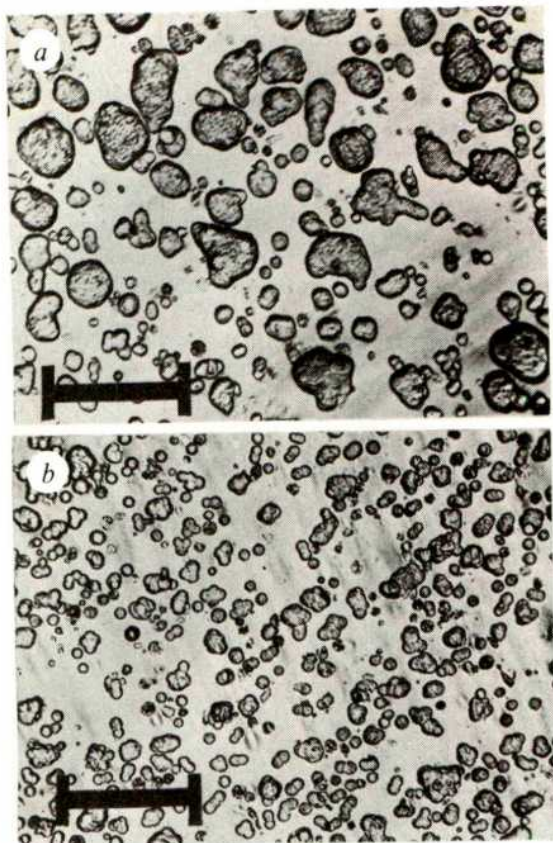


Fig. 2 Photomicrographs of aggregates made from a suspension of cell density  $0.5 \times 10^6$  cells  $\text{ml}^{-1}$ . *a*, Control aggregates; *b*, in the presence of 1 mM db cyclic AMP and 1 mM theophylline. Bar, 200  $\mu\text{m}$ .

(10 mM) or papaverine (1 mM) were each made up in RPMI 1640. The size of the aggregates was measured by placing 0.5 ml of the suspension in a 35-mm plastic Petri dish, and photographs of the aggregates taken. The negative was then projected through an enlarger at a fixed distance. The outlines of the aggregates were traced on to paper and the size of each aggregate measured by comparing with standard metric circle and ellipse templates. The results were then expressed in  $\mu\text{m}$  diameter of cross section of the aggregates. About 300 aggregates were analysed for each determination.

The aggregation of BT-20 over a 24-h period depended on the number of cells present in the incubation. Figure 1 shows that the mean size of aggregates obtained was maximum at about  $0.5 \times 10^6$ – $1.0 \times 10^6$  cells  $\text{ml}^{-1}$ . A 0.5 ml volume of a suspension of this cell density yielded a reasonable number of discrete aggregates for measurement. In the presence of 10% serum the aggregates were slightly larger, but many more cells adhered to glass. Occasionally a thick cord of cells detached from the glass making measurements difficult. Using a standard density of  $5 \times 10^5$ – $10^6$  cells  $\text{ml}^{-1}$ , we found that the addition of 2 mM db cyclic AMP and 2 mM theophylline greatly inhibited the aggregation of BT-20 cells. Figure 2*a* is a photomicrograph of the control aggregates, and Fig. 2*b* shows aggregates obtained in the presence of db cyclic AMP and theophylline. In Fig. 3*a* we present histograms of aggregate size in the control and treated cultures. The figures show that db cyclic AMP and theophylline restricted the diameters of aggregates to less than 25  $\mu\text{m}$ . In Table 1 we show the percentage of aggregates with diameters  $\geq 25 \mu\text{m}$  in the control and treated incubations. After 24 h, db cyclic AMP and theophylline inhibited reaggregation by a factor greater than 4.

The inhibitory effect of db cyclic AMP and theophylline was reversible. After removal of the drugs, the cells and aggregates were washed three times with 10 ml of medium, resuspended in 3 ml of medium, and then incubated on the gyrotary shaker. Comparison of Fig. 3*b* (48-h aggregates) with Fig. 3*a* (24-h aggregates) shows that after the further 24-h incubation there were many more large aggregates and the distribution of the size of aggregates was comparable with that of the control at 24 h. The control aggregates after 48 h of incubation also increased in size as shown in Fig. 3*b*. In another experiment, similar results were obtained when aggregates from treated incubations were washed and trypsinised to yield suspensions of single cells. These cells reaggregated to the same extent as the control cells.

To ascertain that db cyclic AMP and theophylline did not kill BT-20 cells, we carried out the Trypan blue exclusion test after 24 h of incubation. Both treated and control incubations showed the same degree of cell viability ( $>90\%$ ). Single cells and aggregates in treated incubations also attached to glass and spread out after removal of the drugs and reincubation without shaking. The inhibitory effect of db cyclic AMP and theophylline was dependent on concentration. Figure 4 shows the increasing degree of inhibition with increasing concentration of drugs. It is possible that db cyclic AMP was hydrolysed by the cells to butyrate, which has been shown to produce a similar effect on cells as cyclic AMP<sup>19</sup>. We tested the effect of sodium butyrate on BT-20 aggregation, and found that 2 mM butyrate was highly toxic to cells. At a concentration of 0.5 mM, however, toxicity was not detected and there was no effect on cell aggregation. The compound cyclic AMP alone also inhibited aggregation, but a much higher concentration (5–10 mM) was necessary.

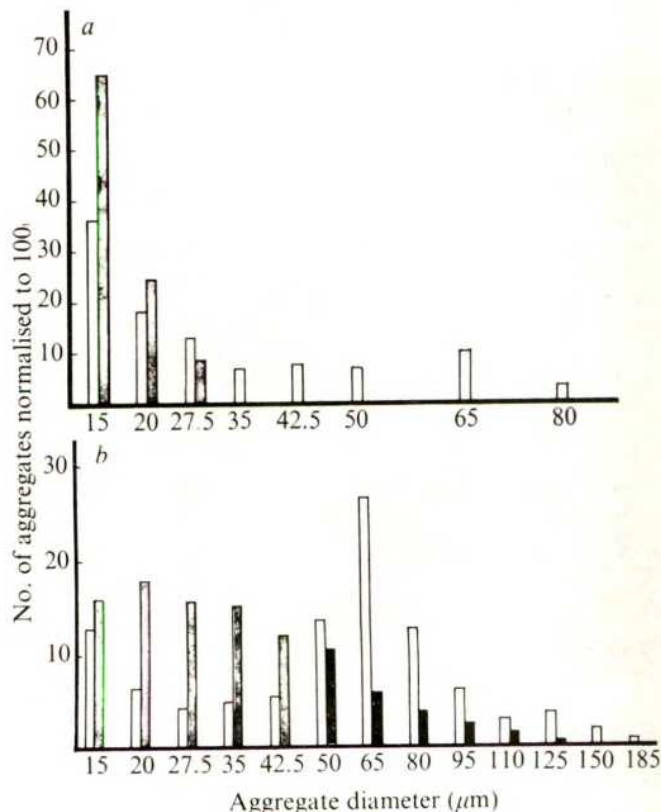


Fig. 3 Histograms showing the effect of db cyclic AMP and theophylline on aggregation of BT-20 cells. *a*, 24-h incubation. Open bars, control aggregates; shaded bars, aggregates treated with db cyclic AMP and theophylline. *b*, 48-h incubation. After the first 24-h incubation the aggregates were collected, spun down, washed, and resuspended in RPMI medium without serum or drugs for the second 24-h incubation. Open bars, control aggregates; shaded bars, aggregates treated with db cyclic AMP and theophylline for 24 h and recovery after removal of the drugs.



**Table 1** Inhibitory effect of db cyclic AMP and theophylline on aggregation of BT-20 cells

	Control	db cyclic AMP + theophylline	Control	Recovery*
	24 h	24 h	48 h	48 h
Aggregates with diameters ≥ 25 µm	46.2	10.7	80.6	66.1

Results are expressed as the percentage of aggregates greater or equal to 25 µm diameter in relation to the total number of aggregates measured.

\*After treatment for 24 h with db cyclic AMP and theophylline, the cells were centrifuged, washed three times with 10 ml of medium, and then resuspended in 3 ml of medium. The aggregates were measured 24 h later.

The compound db cyclic AMP has been shown to be more resistant than cyclic AMP to hydrolysis by phosphodiesterase<sup>20</sup>. We have further tested the effect of theophylline alone on reaggregation, and found that it was almost as effective as the combination of db cyclic AMP and theophylline. Of the other phosphodiesterase inhibitors, we have tested papaverine and trifluoperazine. The latter was very toxic to BT-20 cells at 50 µM. At 20 µM, however, toxicity was not apparent and there was no effect on aggregation. Papaverine, on the other hand, was well tolerated by BT-20 cells. At 100 µM it produced 70–80% inhibition of aggregation without toxicity. (A detailed report on the effect of papaverine on cell–glass and cell–cell adhesion is in preparation.)

The phenomenon of cell aggregation may involve several steps. Orr and Roseman<sup>21</sup> have found that the initial intercellular adhesion of embryonic chick neural retina cells was not affected by cyclic AMP, but Kuroda<sup>22</sup> found that cyclic AMP during 24 h of incubation inhibited the aggregation of embryonic neural retina cells. We have shown in this series of experiments that cyclic AMP and some inhibitors of phosphodiesterase inhibited aggregation of BT-20 cells in suspension. A number of investigators<sup>23–25</sup> have observed that cellular junctions, such as desmosomes, form in aggregates. Some preliminary electron microscopic studies on BT-20 aggregates also showed the formation of desmosomes after 24 h of incubation. We are pursuing the investigation to see if cyclic AMP inhibits the formation of desmosomes.

Several studies<sup>10, 26–29</sup> indicate that aggregation-promoting factors of a glycoprotein nature are involved in a number of systems from sponge cells to rat hepatoma cells. Since

cyclic AMP can influence a number of cell surface activities, it is conceivable that its inhibitory effect on cell aggregation is due to a change in the orientation of these 'aggregation molecules' by an alteration of membrane fluidity, or a direct effect on the biosynthesis of these molecules.

The inhibitory effect of cyclic AMP on membrane activities of human cells has also been noted by Bryant and Sutcliffe<sup>30</sup>, who found that high concentrations of intracellular cyclic AMP inhibited the adhesiveness of human basophils, eosinophils, and neutrophils. Increasing the concentration of cyclic AMP level also inhibits human platelet aggregation<sup>31</sup>.

Our experiments suggest that cyclic AMP, by its modulation of adhesion between carcinoma cells as they form aggregates, may have a role in the propagation of cancer<sup>32</sup>.

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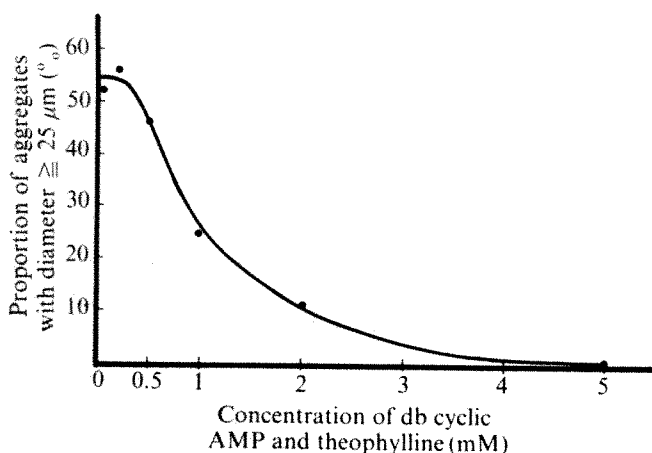
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**Fig. 4** Effect of varying concentrations of db cyclic AMP and theophylline on aggregation of BT-20 cells, which were incubated in 3 ml of medium on a gyrotary shaker. The medium contains db cyclic AMP and theophylline at various concentrations. The sizes of aggregates at 24 h were measured.



## Abortogenic activity of antiserum to alpha-foetoprotein

ALPHA-FOETOPROTEIN (AFP), a glycoprotein of molecular weight 70–72,000, is synthesised by the yolk sac and by perivascular parenchymal cells of the mammalian foetal liver and gastrointestinal tract<sup>1–3</sup>. Increased levels of AFP occur in maternal and foetal sera during pregnancy<sup>4,5</sup>, and indirect evidence suggests some production of AFP by the chorionic tissues<sup>6</sup>. Increase of AFP in amniotic fluid has been correlated with neural tube defects, anencephaly, hydrocephaly, congenital nephrosis and foetal death<sup>7–10</sup>. The specific function of AFP has not been determined, but oestrogen-binding and immunoregulation during pregnancy have been implicated<sup>11,12</sup>. Smith<sup>13</sup> noted an effect of rabbit antiserum to rat AFP when injected into pregnant rats. We now report evidence for a specific abortogenic activity

of rabbit antiserum to murine AFP (anti-AFP) when administered during gestation in the laboratory mouse.

Approximately 100 pregnant mice (day 9–20 of gestation) of the DB2A, C57BL, or Nya:NYLAR strains were injected intraperitoneally with 0.5–2.0 ml of specific anti-AFP (prepared as described under Table 1), with non-immune rabbit serum (NRS), or with buffered saline. The mice were examined 6, 12, 18 and 24 h after injection, and the cages were inspected for aborted fetuses. At 24 h, the mothers were autopsied; the litter size and foetal dimensions were determined. Foetuses and neonates from mothers in all treatment groups were examined for gross morphological changes. Histological sections of the foetal and maternal tissues were prepared using Bouin's fixation and haematoxylin and eosin. The uteri with implantation sites were examined in multiple sections. Samples of maternal liver, ovary, lung, spleen and kidney were examined routinely.

Three levels of response were noted: no effect; partial abortion indicated by subplacental haemorrhage; and complete abortion. Passage of aborted foetuses usually occurred 16–20 h after injection. Anaphylaxis was rarely observed. Of the animals treated with specific anti-AFP, 68% exhibited a physiological response (Table 1). Partial and complete abortions both occurred in the latter two thirds of gestation. Partial abortions tended to occur earlier and complete abortions slightly later. As a rule, the greater the

foetal mass, the more anti-AFP was needed to produce the response. During mid-pregnancy 0.5–1.0 ml of intra-peritoneally administered anti-AFP was sufficient to produce abortion. Towards the end of pregnancy, induction of abortion was less frequently successful and required up to 2.0 ml of anti-AFP.

Gitlin and Koch<sup>17</sup> showed that heterologous IgG readily passes through the placenta in the mouse by both first and second order reactions. Thus, the passage of rabbit anti-AFP antibodies across the mouse placenta is likely. In the pregnant rat, Sell *et al.*<sup>18</sup> and Colquhoun *et al.*<sup>19</sup> reported a steep increase of AFP in maternal serum beginning at days 10–14 and continuing to birth. Sell and Alexander<sup>20</sup> further demonstrated that a catabolic site for AFP is present in the foetus or placenta during the last week of gestation in the rat. These studies indicated that increasing amounts of AFP traverse the placenta as development proceeds. A similar increase of AFP levels in late pregnancy in mice could account for a neutralisation of anti-AFP antibodies at the doses we tested.

The difference in response levels of the three treatment groups was significant by  $\chi^2$  analysis ( $P < 1.0\%$ ). None of the animals injected with buffered saline exhibited physiological or pathological effects. More than 90% of the mice treated with NRS also showed no effect. In the three animals aborted by NRS, the effect occurred on days 15 and 16. This suggested a nonspecific susceptibility of the uterus to extrinsic factors at this time.

To confirm the specificity of the anti-AFP effect, we tested rabbit antisera to whole mouse serum and to three mouse protein antigens that are similar to AFP in either structure or site of origin: albumin, complement (C3) and transferrin<sup>21</sup>. These antisera caused neither partial nor complete abortion in any animal tested (Table 1). As a further test of specificity, aliquots of anti-AFP for injection were absorbed individually with freshly prepared pregnant uterine tissue extracts or with purified AFP fractions obtained from column fractionation. The tissue saline extracts were prepared according to Witebsky's<sup>22</sup> method. Serum specificity was tested by blocking experiments, using both purified AFP antigen and tissue (uterus and spleen) absorption. The tissues from six pregnant mice (13–18 d gestation) were homogenised in phosphate-buffered saline (pH 7.6) and the resultant tissue brei was absorbed in equal proportions (w/v) with the antisera for 30 min at 37 °C, then overnight at 4 °C. Precipitation-inhibition results were confirmed by immunodiffusion. With two exceptions the absorbed antisera produced no effect when injected into pregnant mice at days 13–16 of gestation. Anti-AFP absorbed with a murine spleen tissue extract retained abortogenic activity, and anti-AFP absorbed with one of three uterine tissue samples caused an anaphylactic response.

The mechanism of pregnancy interruption by anti-AFP is unknown, although the specificity of the reaction is evidenced by target tissue absorption and by the lack of effect of rabbit antisera to other mouse serum proteins. Except for intrauterine clot formation, histopathological studies were unrevealing. There seemed to be no inflammatory or lymphocytic infiltrates in the decidua or placenta, and vasculitis was not observed in any tissue. Although studies by Smith<sup>13</sup> demonstrated that the injection of pregnant rats with anti-AFP serum may also result in developmental abnormalities, none was observed in autopsied or surviving litters of any of the mice we treated.

The abortogenic activity of anti-AFP may be due to an anaphylactoid contraction of the uterine smooth muscle; that is, the response may be an *in vivo* correlate of the Schultz-Dale reaction<sup>23</sup>. This humoral mechanism would require the presence of AFP in the uterine or placental tissues. The demonstration by Michel *et al.*<sup>24</sup> of AFP receptors in the immature rat uterus provides some support for this hypothesis.

**Table 1** Response of pregnant mice to intraperitoneally injected rabbit anti-AFP, non-immune rabbit sera (NRS), physiological saline or rabbit antisera to other mouse serum proteins

Treatment group	Response	No. animals	Gestational age (d)* Range	Mode
Anti-AFP	Complete abortion	11/31	12–17	15
	Partial abortion	10/31	9–14	13
	No effect	10/31	14–20	18
NRS	Complete abortion	3/36	15–16	15
	No effect	33/36	9–20	15
Saline	No effect	16/16	9–20	17
Anti-transferrin	No effect	4/4	15–18	17
Anti-complement	No effect	4/4	14–17	15
Anti-albumin	No effect	6/6	12–15	15
Anti-whole mouse sera	No effect	6/6	11–18	14

Rabbit antiserum to murine AFP (anti-AFP) was produced by immunisation with antigen fractions derived from amniotic fluid<sup>14</sup>. The AFP was isolated by gel filtration on Sephadex G-75, followed by shallow-gradient DEAE ion exchange chromatography and preparative gel electrophoresis<sup>15</sup>. Disc gel electrophoresis showed a single band, and after immunoelectrophoresis a single precipitin arc was present in the  $\alpha$ -1 region. In spite of these indications of purity, albumin was detected as a trace contaminant after immunisation and was eliminated by absorption using two parts normal mouse sera to eight parts antiserum. The absorbed anti-AFP was immunoreactive to mouse AFP as evidenced by single-band formation after immunodiffusion and counterimmunoelectrophoresis. Anti-AFP from four rabbits were used. These antisera had specific antibody concentrations of 0.424–0.532 mg ml<sup>-1</sup> as determined by the quantitative precipitin reaction. Each anti-AFP serum was tested at all stages of pregnancy in separate experiments. The results included in Table 1 summarise all these data. Control sera consisted of non-immune rabbit serum (NRS), rabbit anti-whole mouse sera, rabbit anti-mouse albumin, rabbit anti-mouse transferrin and rabbit anti-mouse complement. The NRS serum was absorbed with mouse sera as described above. The anti-albumin serum was prepared from mouse albumin isolated by ion exchange chromatography; the antisera to whole mouse sera, transferrin and complement were obtained from Microbiological Associates. The specificity of these control sera was confirmed both by immunoelectrophoresis and immunodiffusion using whole and fractionated mouse serum. The immunological activity of each of the four anti-AFP and of the control antisera was attributed to the IgG, as determined by immunoelectrophoresis. The anti-AFP and anti-albumin sera were obtained from hyper-immunised rabbits bled 6 weeks after the first injection thus assuring a predominance of IgG antibody in the serum.

\*Length of gestation at time of injection. Gestational age was estimated by palpation, and foetal size was determined by crown-rump measurements according to Rugh<sup>16</sup>.



An alternative possibility is suggested by the work of Murgita and Tomasi<sup>22,25</sup>, who demonstrated that AFP suppresses both the primary and secondary antibody response to sheep red blood cells without inducing cytotoxic effects *in vitro*. Further studies by Murgita and Tomasi revealed that AFP can suppress allogeneic and mitogen-induced lymphocyte transformations (T-cell dependent functions) in mice<sup>25</sup>. Finally, immunofluorescence studies by Dattwyler *et al.*<sup>26</sup> strongly suggest the presence of AFP receptors on the surface of certain T-cell lymphocyte populations in mice. On the basis of these findings, it can be postulated that AFP is instrumental in maintaining pregnancy, the foetus being viewed as an allograft in the maternal tissues. Extrapolating this hypothesis, neutralisation of AFP at the maternal-placental interface might conceivably initiate rejection of the foetus through a cell-mediated immune mechanism. Although evidence of a graft (foetus) rejection was not histologically apparent, the physiological release by lymphocytes of vasoactive lymphokines, such as prostaglandins<sup>27</sup>, and/or the release of T-cell suppressor function might not necessarily leave a morphological trace.

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## Early production of intracellular IgM by B-lymphocyte precursors in mouse

IN BALB/c mice, surface immunoglobulin (Ig)-bearing B lymphocytes are first detectable by immunofluorescence at 17 d gestation in the liver and spleen<sup>1</sup>. Explants of 14-d foetal liver<sup>1</sup> and spleen<sup>2</sup>, and 15-d bone marrow (our unpublished observations), have been shown to generate Ig-bearing B cells *in vitro* after 4-7 d of culture, suggesting that B lymphocytes normally develop multifocally in the haemopoietic tissues of mice. We have used direct immunofluorescence to demonstrate cells with intracellular IgM and no detectable surface Ig in mouse foetal liver as early as 12 d gestation. Our results suggest that B lymphocyte precursors synthesise IgM several days before they incorporate these molecules into their plasma membranes as cell-surface receptors for antigen.

Purified goat antibodies to mouse  $\kappa$  chains (anti- $\kappa$ ),  $\mu$  chains (anti- $\mu$ ),  $\alpha$  chains (anti- $\alpha$ ) and  $\gamma 2a$  and  $\gamma 2b$  chains (anti- $\gamma 2$ ) were raised, adsorbed and purified on various immunoadsor-

bants, and tested for specificity, as previously described<sup>3-5</sup>. While the anti- $\gamma 2$  antibodies used in our original experiments on the development of B cells in foetal liver explants<sup>1</sup> were subsequently found to have contaminating antibodies against  $\kappa$  chains (probably accounting for most, and possibly all, of the apparent  $\gamma 2$ -bearing cells seen), the anti- $\gamma 2$  (as well as the anti- $\mu$  and anti- $\alpha$ ) antibodies used in the experiments reported here could not be demonstrated to have any anti- $\kappa$  activity, and were class specific by Ouchterlony analysis, immunofluorescence staining of fixed cell preparations of various mouse myelomas, and modulation experiments in which each antibody preparation did not modulate surface Ig of other classes at a concentration of 1 mg ml<sup>-1</sup>. The purified antibodies were conjugated to fluorescein or rhodamine as previously described<sup>3,4</sup>. In some experiments purified polyspecific goat anti-mouse Ig conjugated to fluorescein (anti-MIg-FI) was used<sup>6</sup>. Nonspecific staining of fixed cells was eliminated by adsorbing the conjugates with mouse liver powder. The conjugated antibodies were used at a final concentration of 500  $\mu$ g ml<sup>-1</sup>.

Table 1 Ontogeny of IgM<sup>+</sup> cells in BALB/c foetal liver\*

Gestation (d)	Surface IgM <sup>+</sup> cells (%)†	Intracellular IgM <sup>+</sup> /surface IgM <sup>-</sup> cells (%)‡
12	0.0	0.03
13	0.0, 0.0, 0.0, 0.0	0.015, 0.0, 0.007, 0.0, 0.03, 0
14	0.0, 0.0, 0	0.33, 0.05, 0.0, 0.05
15	0.0, 0	0.02, 0.1, 0.06
16	0.0	0.32, 0.2
17	0.15, 0.10	0.75, 2.0
18	0.6	2.8
19	1.1	4.2

\*Cells were stained in suspension with anti- $\mu$ -Rd to label surface IgM and with anti- $\mu$ -FI (after cytocentrifugation and fixation) to label intracellular IgM. As discussed in the text, surface IgM<sup>+</sup> cells stained with both rhodamine and fluorescein conjugates while intracellular IgM<sup>+</sup>/surface IgM<sup>-</sup> cells stained only with fluorescein. Usually, more than 50,000 cells were scanned.

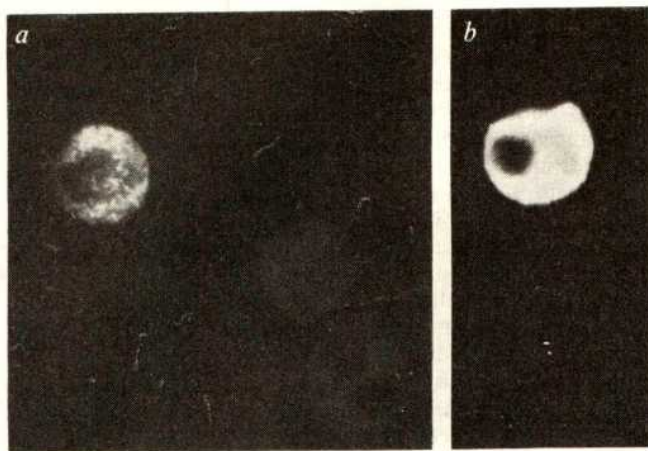
†Each number represents one experiment.

‡These cells also failed to show surface labelling with polyspecific anti-MIg, suggesting the lack of any detectable surface Ig.

Haemopoietic and lymphoid tissues were removed from BALB/c embryos of various ages (the day of appearance of a vaginal plug being taken as day 0), newborns or 6-week-old adults, and dissociated into single cell suspension in Dulbecco's modified Eagle's medium containing 15 mM HEPES, 0.2% sodium azide and 10% foetal calf serum (DMEM). The cells were labelled in suspension with rhodamine-conjugated anti-Ig antibodies at 0 °C for 30 min to demonstrate surface Ig, washed and cytocentrifuged onto a glass slide, fixed in cold 5% acetic acid in ethanol, and stained for intracellular IgM with fluorescein-conjugated anti-Ig antibodies at 37 °C for 25 min. After thorough washing, the cells were examined under a coverslip for surface (rhodamine) and intracellular (fluorescein) fluorescence, using a Zeiss Ultraphot II, or a Leitz Ortholux microscope, equipped with phase contrast and incidence fluorescence optics and an Osram HBO 200 mercury arc lamp. Fluorescein-conjugated anti-Ig antibodies for surface staining and rhodamine conjugates for intracellular staining gave the same results.

As has previously been reported<sup>1</sup>, cells in foetal liver with detectable IgM on their surface were first seen at 17 d gestation (Table 1). But foetal liver cells with readily detectable intracellular IgM and without detectable surface Ig were first seen at 12 d in some mice and by 15 d in all litters studied (Fig. 1). Although most of these cells were large (comparable in size with medium or large lymphocytes), some were the size of small lymphocytes. The staining of fixed cells with fluoresceinated-anti- $\kappa$  antibodies (anti- $\kappa$ -FI) gave similar results to those obtained with anti- $\mu$ -FI, but no intracellular labelling was seen with anti- $\gamma 2$ -FI or anti- $\alpha$ -FI. This attested to the specificity of the intracellular labelling with anti- $\mu$ -FI and anti- $\kappa$ -FI, and indicated that these intracellular IgM<sup>+</sup>/surface Ig<sup>-</sup> cells





**Fig. 1** *a*, Cell stained for intracellular IgM with anti- $\mu$ -Rd in 15-d foetal liver; *b*, For comparison, a plasma cell in adult spleen stained for intracellular IgM with anti- $\mu$ -Rd. The film was exposed for 3.5 min in *a* and 2 s in *b* ( $\times 300$ ).

produced both heavy ( $\mu$ ) and light Ig chains. Nonspecific labelling due to binding to Fc receptors was also excluded by showing that  $F(ab')_2$  fragments of anti- $\mu$  antibodies, conjugated to fluorescein, gave results similar to those obtained with undigested anti- $\mu$ -Fl.

To detect intracellular Ig, cells must be fixed before labelling, while cell surface Ig can be demonstrated by labelling either living cells in suspension or cells fixed on a slide. Thus, cells with surface Ig (B cells) stained with both fluorescein and rhodamine in our double labelling technique, whether or not they had detectable intracellular Ig. Therefore, to determine whether these cells also had detectable intracellular Ig, we labelled surface IgM on living adult spleen cells with anti- $\mu$ -Rd in capping conditions<sup>7</sup> (DMEM without azide for 20 min at 20 °C) and then looked for intracellular IgM with anti- $\mu$ -Fl, after fixation. All the fluorescein labelling coincided with the rhodamine labelling in the caps and was not seen elsewhere in the cell, indicating that most surface-IgM<sup>+</sup> cells in spleen did not have intracellular IgM detectable by this means. In a similar experiment with newborn liver cells, a small proportion of the surface IgM<sup>+</sup> cells also showed intracellular IgM.

As Table 2 shows, intracellular IgM<sup>+</sup>/surface Ig<sup>+</sup> cells were found only at sites of B lymphocyte production, such as foetal and newborn liver<sup>1</sup> and spleen<sup>2</sup> and adult bone marrow<sup>7,9</sup>. They were not found in adult lymph node or spleen, or in foetal thymus. Their distribution, and the fact that they synthesise Ig, strongly suggest that they are B lymphocyte precursors, and we will refer to them subsequently as pre-B cells. IgM-containing plasma cells and other IgM-secreting lymphoid cells in adult lymphoid tissues, were readily distinguished from pre-B cells because the former stained much more intensely with anti- $\mu$ -Fl (Fig. 1) and always had readily detectable IgM on their surface. Table 2 shows that most IgM-synthesising cells in foetal and newborn liver and spleen, and in adult bone marrow are pre-B cells rather than B cells. Since they appear several days earlier in ontogeny than do B cells with surface Ig, one should be able to use fluorochrome-labelled antigens to study the generation

of antibody diversity earlier than previously possible. In addition, having an intracellular marker for pre-B cells should be useful for studying B-cell development in normal and immunodeficient animals, including man.

The pre-B cells in adult bone marrow described here presumably are the same as, or overlap considerably with, the surface Ig<sup>+</sup> cells, previously described in bone marrow, which can differentiate into surface Ig<sup>+</sup> B cells *in vitro* and *in vivo*<sup>8,9</sup>. Our failure to detect significant numbers of pre-B cells in the adult spleen suggests that this organ is not an important site where pre-B cells differentiate to B cells in adult animals, and that most "null cells" (cells lacking T or B cell surface markers)<sup>6,10</sup> in the spleen probably do not belong to the B-cell lineage. It also seems likely that our pre-B cells in foetal liver are among the "Type 1 B cells" described by Melchers *et al.*<sup>11</sup> as large cells in 10–12-d mouse embryos and in 13–15-d foetal liver and in adult bone marrow, which incorporate radioactive leucine into rapidly turning over ( $T_{1/2}$  = 1–3 h) 7–8S IgM. Since such 7–8S IgM could be labelled by lactoperoxidase-catalysed radioiodination of adult bone marrow cells, Melchers *et al.* suggested that it is on the cell surface, and that the failure of others to demonstrate it there may be due to its rapid turnover<sup>11</sup>. This is an important question, as the presence or absence of Ig at the cell surface would determine whether or not such cells could bind, and possibly respond to antigen.

**Table 3** IgM<sup>+</sup> cells in foetal liver explants inhibited with anti- $\mu$  antibody

Age of liver (d)	Days in culture	Anti- $\mu$ in culture ( $\mu$ g ml <sup>-1</sup> )	Surface IgM <sup>+</sup> cells (%)	Intracellular IgM <sup>+</sup> /surface IgM <sup>+</sup> cells (%)
18	3	0	1.8	2.9
18	3	20	0	3.2
14	7	0	7.4	9.5
14	7	20	0	7.2

To help resolve this apparent discrepancy, we cultured foetal liver fragments on top of Millipore filters as described previously<sup>1</sup> in the presence or absence of unconjugated anti- $\mu$  antibodies (20  $\mu$ g ml<sup>-1</sup>). We have shown previously that anti- $\mu$  antibodies in such cultures induce the disappearance (and prevent the development) of cell-surface IgM<sup>5</sup>. As Table 3 shows, although no surface IgM was found on anti- $\mu$  treated cells this treatment did not affect the proportion of cells with intracellular IgM or the intensity of their staining. Thus most of the IgM that we have detected as intracellular in pre-B cells by our double labelling technique seems not to be available at the cell surface, and unlike B cells<sup>5</sup>, these cells are not killed or suppressed by prolonged exposure to anti-Ig antibodies. These experiments do not exclude the possibility that a small proportion of the IgM synthesised by these cells is exposed at the cell surface.

The biological significance of the intracellular IgM in pre-B cells is enigmatic. It seems more likely that it is destined for the plasma membrane than for secretion, but then it is not clear why there is such a long period between synthesis and incorporation into the cell surface. It may be that it provides a mechanism for protecting these cells from interacting with antigen during a period of clonal expansion and/or diversification of activated V genes. If the IgM is destined for the plasma membrane, the failure to find detectable intracellular IgM in B cells with surface IgM, suggests that it may accumulate in pre-B cells because it cannot get to, or be stably incorporated into, the plasma membrane. Perhaps, this incorporation must await the production of an appropriate glycosyl-transferase, proteolytic enzyme, or cell-surface acceptor protein. Whatever the explanation, these cells may provide a useful system for studying where membrane proteins are made and how they are distributed to their appropriate location in the cell.

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**Table 2** Distribution of IgM<sup>+</sup> cells in various BALB/c tissues

Tissue	Age	Surface IgM <sup>+</sup> Cells (%)	Intracellular IgM <sup>+</sup> /surface IgM <sup>+</sup> cells (%)
Foetal thymus	14 or 17 d	<0.01	<0.01
Spleen	Newborn	3.9	4.9
Spleen	6 weeks	31	<0.1
Lymph nodes	6 weeks	18	<0.1
Bone marrow	6 weeks	7	12
Liver	Newborn	1.4	3.3



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## Resistance of activated macrophages to H-2 antibody-mediated cytotoxicity and Fc rosette inhibition

MACROPHAGES can have critical roles in repair, inflammation and a large variety of immunological phenomena<sup>1,2</sup>. The biological roles of these cells often occur subsequent to their 'activation', a subjective term which refers to a greatly heightened state of reactivity<sup>1-3</sup>. Activation can be induced by a variety of stimuli including ingestion of certain macro-organisms, contact with immune complexes, bacterial lipopolysaccharides and lymphokines<sup>3</sup>. Activated macrophages usually show an increase in cell size, increased adherence properties and cellular processes, lysosomal enzyme activity, and phagocytic ability<sup>1,2</sup>. In addition, alteration in characteristics and functions of various macrophage receptors can apparently take place as a consequence of activation<sup>4,5</sup>. Such a change is described in this paper: it involves Fc receptors as detected by Fc or EA rosette formation (see Table 1), and their relationship to surface mouse major histocompatibility (H-2) antigens as detected by anti-H-2 alloantisera. Aside from the general relevance this change has with regard to membrane alterations which occur in activated lymphoreticular cells, it can also be exploited as a marker for activated macrophages.

Recent results have shown that formation of Fc (EA) rosettes between IgG antibody-coated erythrocytes and lymphoid cells bearing Fc receptors can be totally blocked by preincubation of the lymphoid cells with relevant anti-erythrocyte against the major histocompatibility antigen complex<sup>6,7</sup>. An example of this is seen in Table 1: preincubation of DBA/2 (H-2<sup>d</sup>) mouse strain spleen cells with anti-H-2<sup>d</sup> alloantisera resulted in a marked reduction of the ability of the cells to subsequently form Fc rosettes with IgG antibody-coated sheep erythrocytes (SRBCs) compared with normal mouse serum (NMS)-treated controls. Preincubation with irrelevant anti-H-2 serum (for example anti-H-2<sup>b</sup>) resulted in no inhibition. When normal peritoneal exudate cells (PECs), containing an average of about 24% macrophages (assessed by ingestion of latex particles) were

similarly tested, rather drastic inhibition in the ability of the cells to form Fc rosettes was once again noted. Since macrophages bear the Fc receptor<sup>1,4</sup>, these results show that the Fc rosetting ability of most or all normal (unstimulated) macrophages is inhibited by the anti-H-2 pretreatment.

Quite different results were obtained, however, when PECs from BCG-injected or proteose-peptone-injected mice were tested; such injections result in 'activation' of the macrophage population by a variety of criteria<sup>2,5</sup>, and, as shown in Table 1, such activated PEC populations retained a high degree of their Fc rosetting ability after anti-H-2 serum pretreatment. Equally significant, the proportions of phagocytic cells and anti-H-2-resistant Fc rosettes in these cell suspensions were almost equal (Table 1). It thus seems that it is the activated macrophages which are resistant to the inhibiting effects of the anti-H-2 serum. This was verified by testing plastic-adherent (macrophage-rich) compared with non-adherent (lymphocyte-rich) cells from these cell suspensions: as shown in Table 1, the adherent cells were only minimally inhibited, whereas non-adherent cells were quite severely impaired in their ability to form Fc rosettes.

Activated (blast) lymphocytes, either B cells mitogenically transformed with *Escherichia coli* lipopolysaccharide<sup>8</sup> (LPS) or T cells activated to allogeneic antigens *in vivo*,

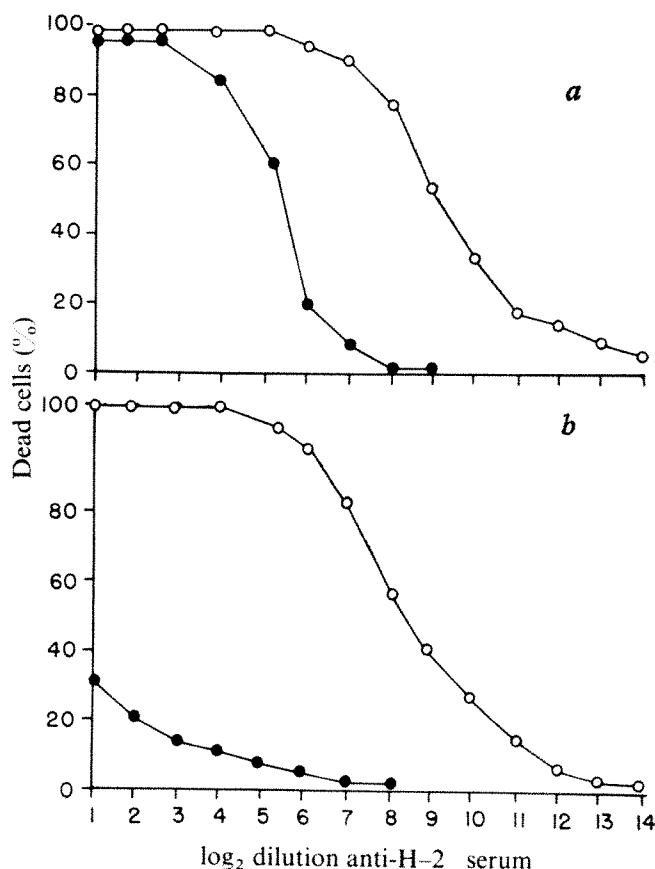


Fig. 1 Cytotoxicity of CBA anti-DBA/2 (anti-H-2<sup>d</sup>) serum on DBA/2 spleen (a) or peritoneal exudate cells (PEC) from peptone-stimulated mice (b), using GPC or RC as sources of complement. Dead cells were assessed by Trypan blue uptake<sup>20</sup>. The PEC population contained about 65% phagocytic cells before testing. The GPC (●) and RC (○) were both purchased from Gibco in a lyophilised form. The GPC was used at a dilution of 1:6. The RC was first extensively absorbed with mouse RBCs, spleen, thymus and various mouse tumour culture cell lines to remove naturally occurring antibodies against mouse nucleated cells. It was then used at a dilution of 1:2. The cytotoxic curve of anti-H-2<sup>d</sup> serum on normal PECs was very similar to that found with spleen. These experiments were repeated many times using different sources of GPC, including fresh guinea pig serum, and different batches of anti-H-2<sup>d</sup> serum, the same results being obtained.

**Table 1** Effect of anti-H-2 serum preincubation on the ability of various cell populations to form Fc rosettes\*

Cells tested	% Phagocytic cells	% Fc rosettes obtained after NMS	% Fc rosettes obtained after Anti-H-2 <sup>d</sup>
Spleen	4.4±2.1	47.6±6.0	2.8±1.5
Normal PECs	24.4±8.3	54.0±9.7	3.1±1.6
BCG-activated PECs	31.0±19.6	64.2±15.4	27.0±14.7
Peptone-activated PECs	44.1±12.9	83.3±4.6	47.0±8.2
Adherent activated PECs	79.8±6.6	89.7±5.8	82.5±5.8
Non-adherent activated PECs	5.1±2.0	36.3±8.4	4.3±2.8
GvH spleen†	3.2±0.95	46.0±6.2	3.0±1.1
LPS spleen cells‡	3.5±2.1	44.2±8.9	2.0±0.8
P815 mastocytoma § cell line (H-2 <sup>d</sup> )	0	67.2±35.9	0
L1210 myeloid leukaemia cell line (H-2 <sup>d</sup> )	0	42.7±14.4	0
FVL erythroleukaemia cell line (H-2 <sup>d</sup> )	0	33.5±16.2	0
P388D <sub>1</sub> macrophage-like cell line (H-2 <sup>d</sup> )	61.5±15.6	95.2±2.0	84.2±11.8
SaD/2 solid tumour ¶	10.8±4.6	25.0±8.2	12.5±4.2
SaD/2 solid tumour    (iron-treated)	1.1	14.0	1.8

\*Fc rosettes were assessed using SRBCs coated with a 1/100 (subagglutinating) dilution of a heat-inactivated rabbit hyperimmune anti-SRBC serum, as described previously<sup>12</sup>. Also previously described<sup>12</sup>: the Fc rosette assay, preparation and properties of the anti-H-2<sup>d</sup> (CBA anti-DBA/2) serum, and preparation of the various DBA/2 strain cell suspensions. The inhibitory effect of anti-H-2<sup>d</sup> serum was assessed by preincubating  $0.5 \times 10^6$  cells, previously pelleted by centrifugation with 0.1 ml anti-H-2<sup>d</sup> serum or NMS (normal CBA mouse serum) for 20 min, centrifugation of the cells followed by removal of the serum and addition of 0.5 ml of a 1% suspension of the antibody-coated SRBCs. Rosettes were then made and counted using crystal violet to stain the rosetted cells<sup>12</sup>. Phagocytic cells were assessed by ingestion of latex beads<sup>22</sup> or the osmotic lysis technique<sup>12,23</sup>. The latex method was found to be more reliable when assessing normal macrophages. Selection of adherent and non-adherent PECs was as described previously<sup>12</sup>. The results recorded represent the mean of 4–6 experiments ± standard deviation.

‡B cells were activated in culture by the mitogen *E. coli* LPS<sup>9</sup> as described previously<sup>24</sup> and were collected after 3 d. Such cultures contained about 50% 'blast' cells of which over half were Fc receptor-positive.

†Graft versus host (GvH) spleen refers to spleen cells from lethally irradiated (850 R) C<sub>3</sub>D<sub>2</sub>F<sub>1</sub> mice injected with  $50 \times 10^6$  DBA/2 spleen cells beforehand to obtain activated T cells<sup>19</sup>.

§The P815 line as well as the others are long term established tissue culture cell lines. The P388D<sub>1</sub> cells<sup>10</sup> were provided by Dr H. Koren, National Institutes of Health. The FVL (Friend virus leukaemia) cells were donated by Dr J. C. Kennedy, Queen's University, Department of Pathology. Cells were taken from both stationary and log-phase cultures which account for part of the variability in Fc rosette counts after NMS.

¶DBA/2 mice were injected with  $10^6$  SaD/2 tumour cells, the tumours removed 3–4 weeks later and a single cell suspension made as described previously<sup>12</sup>.

||The SaD/2 tumour cell suspensions were treated with iron filings and magnetism as described previously<sup>12</sup> to remove macrophages. Mean of two experiments recorded.

some of which may be Fc receptor-positive<sup>9</sup>, behaved like their normal counterparts with regard to anti-H-2-mediated inhibition of Fc rosette formation (Table 1).

Thus, activated macrophages, in contrast to normal macrophages, normal lymphocytes and activated lymphocytes are capable of forming Fc rosettes after treatment with relevant anti-H-2 serum. These animal experiments can be mimicked using cells from long term established tissue culture lines which are Fc receptor positive. Whereas cells from the L1210 myeloid leukaemia, a Friend-virus-induced erythroleukaemia, and the P815 mastocytoma were all totally inhibited, the P388D<sub>1</sub> cell line—which is a macrophage-like cell line<sup>10</sup>—retained its full Fc rosetting ability (Table 1).

The possibility of exploiting the observation that activated macrophages can still form Fc rosettes after anti-H-2 treatment as a 'marker' to screen cell suspensions for their presence would seem verified by further experiments (Table 1) in which cell suspensions made from solid fibrosarcoma tumours—which are often known to contain many macrophages<sup>11,12</sup> which can be in an activated state<sup>13</sup>—were tested for the presence of phagocytic cells and anti-H-2 resistant Fc rosette-forming cells. The respective proportions of these cells were very similar, and previous removal of phagocytic cells from the tumour cell suspensions left a residual population of Fc rosette-forming cells which were capable of being totally inhibited by anti-H-2 serum pretreatment.

The reasons why activated macrophages retain their Fc rosetting ability after anti-H-2 treatment are not clear. Some possibilities include: a possible significant drop in the relative density of H-2 antigens on macrophages when they become activated; the number and avidity of Fc receptors are both known to increase on macrophages when they become activated<sup>4,14</sup>; an antigen, other than H-2, to which antibodies in the polyspecific anti-H-2 sera can react, may be present on normal macrophages but not on activated macrophages, thus effectively diluting the potency of the anti-H-2 sera. If this is the case, the Ia

antigens may be involved, as these are present on normal macrophages<sup>15,16</sup> but may not be on activated macrophages<sup>17</sup>.

The first possibility may explain why activated macrophages seem relatively resistant to the cytotoxic effects of anti-H-2 serum and guinea pig complement<sup>12</sup> (GPC). As shown in Fig. 1, killing of such cells requires a much more potent source of complement (in this case rabbit serum<sup>18</sup>) for the cells to be killed. In contrast, normal lymphocytes are easily killed by anti-H-2 serum and GPC, as are activated lymphoid cells<sup>19,21</sup>. These results indicate caution should be exercised when using anti-H-2 sera and GPC as cytotoxic markers on lymphoreticular cell populations as one may inadvertently select for any activated macrophages that may be present.

In summary, it has been found that activation of macrophages is accompanied by an increased resistance of these cells to anti-H-2 serum mediated cytotoxicity and inhibition of Fc (EA) rosette formation; the latter observation can apparently be applied successfully as a simple marker to detect activated macrophages.

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## H-2-like specificities of foreign haplotypes appearing on a mouse sarcoma after vaccinia virus infection

ATTENTION has focused recently on the interaction of viruses and histocompatibility antigens, and it has been proposed that this relationship may be central to immune surveillance<sup>1-3</sup>. We report here the new expression of H-2 specificities apparently of other haplotypes on the surface of a chemically induced mouse tumour cell line following vaccinia virus infection.

A sarcoma induced by 3-methylcholanthrene (Meth A, H-2<sup>d</sup>) was passaged in syngeneic mice (BALB/c, H-2<sup>d</sup>). At the time of passage, the recipients were also infected intraperitoneally with vaccinia virus. Control BALB/c mice received tumour but no virus. After 7 d tumour cells were taken from the peritoneal cavity from both infected and control mice, washed and tested for a wide range of H-2 antigenic specificities—both public and private. We used a new microradioassay that involves antibody-complement treatment of the target tumour cells followed by testing for the uptake of <sup>14</sup>C-uridine in a postlabelling assay (manuscript in preparation).

We found the normal H-2<sup>d</sup> haplotype specificities in the control tumour (private H-2D.4, H-2K.31 and public H-2 3,8,28, and 35), whereas in the tumour from infected animals we repeatedly and reproducibly found reactions with anti-H-2 sera of restricted specificity apparently detecting ten additional private and three public specificities—normally present only in mice of other H-2 haplotypes (Table 1). The extra-reactivities seemed to be restricted to anti-H-2 sera, since normal mouse sera from various strains, antisera against non-H-2 antigens (Thy 1.2; Ly 4.2) and, interestingly, antisera against I-region-associated (Ia) antigens did not react. The extra specificities were found 5-7 d after infection but after 2 weeks there was no difference between tumour cells from infected and control animals. This time course roughly parallels that of the

**Table 1** Cytotoxic activity of anti H-2 sera against Meth A sarcoma cells grown in syngeneic mice in the absence (control) or presence of vaccinia virus

Pretreatment of tumour cells with Sera*			Meth A control		Meth A passaged with vaccinia virus	
(Haplotype)			<sup>14</sup> C-uridine uptake c.p.m. ± s.d.	% Reduction	<sup>14</sup> C-uridine uptake c.p.m. ± s.d.	% Reduction
—	—	—	3.861 ± 181		3.364 ± 169	
—	—	+	3.641 ± 125		3.790 ± 160	
<b>Private</b>						
anti H-2D.2	(b,g,h,j)	+	3.800 ± 39	0	2.577 ± 361	32
anti H-2D.4†	(a,d,i,t,u,y)	+	176 ± 50	96	131 ± 28	97
anti H-2.9	(f)	+	3.685 ± 143	0	3.827 ± 215	0
anti H-2D.12	(s)	+	2.366 ± 88	35	607 ± 31	84
anti H-2K.15	(j)	+	1.383 ± 94	62	589 ± 81	85
anti H-2.16	(p)	+	1.379 ± 120	62	733 ± 245	81
anti H-2K.17	(q,y)	+	2.427 ± 81	34	1.041 ± 170	73
anti H-2.18	(r)	+	2.459 ± 134	32	3.030 ± 245	21
anti H-2.19	(s,t)	+	3.614 ± 451	0	833 ± 115	78
anti H-2K.20	(u)	+	3.699 ± 188	0	3.982 ± 417	0
anti H-2K.23	(a,h,k,m)	+	3.660 ± 81	0	1.326 ± 381	65
anti H-2D.30	(m,q,v)	+	3.735 ± 53	0	947 ± 120	75
anti H-2K.31†	(d,g,o)	+	423 ± 171	90	141 ± 14	96
anti H-2D.32	(k,o)	+	3.648 ± 280	0	683 ± 77	82
anti H-2K.33	(b,i)	+	4.035 ± 55	0	1.749 ± 251	54
<b>Public</b>						
anti H-2.1		+	3.604 ± 231	0	580 ± 62	88
anti H-2.3†		+	1.529 ± 183	58	120 ± 17	97
anti H-2.5		+	3.880 ± 371	0	1.630 ± 313	57
anti H-2.8†		+	315 ± 32	92	97 ± 32	98
anti H-2.11		+	2.912 ± 192	20	760 ± 92	80
anti H-2.13†		+	112 ± 37	98	121 ± 38	97
anti H-2.25		+	3.622 ± 431	0	3.947 ± 428	0
anti H-2.28†		+	132 ± 44	98	93 ± 17	98
anti H-2.35†		+	151 ± 30	98	108 ± 35	98
<b>Others</b>						
normal mouse serum		+	3.727 ± 91	0	3.401 ± 411	15
anti-Thy 1.2		+	2.953 ± 10	19	3.011 ± 110	20
anti-Ly 4.2		+	3.551 ± 184	0	3.677 ± 315	3
anti-Ia (A.TH anti A.TL)		+	3.042 ± 201	15	3.127 ± 414	27

5 × 10<sup>6</sup> Meth A tumour cells were injected with or without vaccinia virus (10<sup>7</sup> infectious units of Lister strain) into BALB/c mice. 7 d later the tumour cells from both groups were collected from the peritoneum and washed. 2 × 10<sup>4</sup> macrophage-depleted Meth A tumour cells (infected or control) in 50 µl culture medium were incubated in round-bottom wells of sterile microtitre plates with 1 µl of antiserum for 1 h at 4 °C and then 30 min with 50 µl of prediluted (1/10) rabbit complement at room temperature (C°). 10 µl <sup>14</sup>C-uridine (0.3 µCi ml<sup>-1</sup>; 402 mCi mmol<sup>-1</sup>) were added to the plates for a further 16 h. The cultures were collected in a semiautomatic machine. The results are expressed as the percentage reduction in uridine uptake in test samples compared with control samples with complement and no antibody. Similar results were obtained by measuring <sup>14</sup>C-thymidine uptake. The results were also reproduced in a conventional microcytotoxicity titration using serially diluted antisera.

\*The anti-H-2 sera represent sera of the D series raised under contract by the Transplantation Immunology Branch of the National Institutes of Health. For details see National Institutes of Health catalogue and supplements<sup>23</sup>, anti H-2D.12 and anti H-2.11 reacted weakly with normal Meth A tumour cells and not with H-2<sup>d</sup> lymphocytes. Anti H-2K.15, H-2K.17 and H-2.18 seem to contain an unidentified antibody which reacted weakly with H-2<sup>d</sup> lymphocytes. Anti H-2.16 also contains anti 34 which could explain the cytotoxicity against Meth A control. A TH anti-A.TL was obtained from Dr Chella David, the other sera were raised in the London Hospital Medical College.

†Private specificities present on H-2<sup>d</sup> lymphocytes: H-2D.4, H-2K.31; public: H-2.3, 8, 13, 28, 35 (ref. 22).

**Table 2** Absorption of cytotoxic anti-tumour activity from anti-H-2 sera by normal lymphocytes carrying the appropriate H-2 antigenic determinants

Antisera	Absorbing cells* from	Meth A control		% Reduction of <sup>14</sup> C-uridine† uptake by Meth A + vaccinia		Gardener	
		Predicted result	(H-2 <sup>d</sup> )	Predicted result	(H-2 <sup>d</sup> )	Predicted result	(H-2 <sup>k</sup> )
Anti-H-2D.4	—	+	96	+	95	—	2
Anti-H-2D.4	B10.BR (H-2 <sup>k</sup> ; D.4 neg.)	+	96	+	93	—	0
Anti-H-2D.4	B10.A (H-2 <sup>a</sup> ; D.4 pos.)	—	6	—	0	—	0
Anti-H-2D.32	—	—	0	+	82	+	89
Anti-H-2D.32	B10.A (H-2 <sup>a</sup> ; D.32 neg.)	—	0	+	75	—	87
Anti-H-2D.32	B10.BR (H-2 <sup>k</sup> ; D.32 pos.)	—	0	—	9	+	4
Anti-H-2.1	—	—	0	+	88	+	89
Anti-H-2.1	B10 (H-2 <sup>b</sup> ; 1 neg.)	—	0	+	87	+	91
Anti-H-2.1	B10.A (H-2 <sup>a</sup> ; 1 pos.)	—	0	—	18	—	14

\*100 µl of 1/10 diluted antiserum was absorbed with 10<sup>8</sup> spleen cells from B10, B10.BR or B10.A mice for 1 h at 4 °C and tested in presence of complement for cytotoxic activity against normal Meth A, Meth A from vaccinia-infected mice (day 7) and Gardener lymphoma. The tumour cells were then postlabelled by culturing them overnight with <sup>14</sup>C-uridine

†<sup>14</sup>C-uridine uptake in the controls (treated with complement alone) was for Meth A 3.114 ± 251, for Meth A + vaccinia 2.296 ± 132 and for Gardener lymphoma 2.316 ± 613.

induction of cytotoxic T cells against vaccinia-infected syngeneic target cells<sup>4</sup>.

The results in Table 2 show that the extra-reactivity with anti-H-2D.32, directed against a private specificity of the H-2<sup>k</sup> haplotype, could be absorbed with spleen cells from B10.BR (H-2<sup>k</sup>, D32 positive) but not from B10.A (H-2<sup>a</sup>, D.32 negative) mice. Furthermore, cytotoxic activity of anti-H-2.1 could be absorbed with cells from B10.A (positive for 1, a public specificity) but not from C57BL/10 (B10, negative for 1). But we also had experiments in which there was nonspecific removal of activity by normal lymphoid cells. It is unlikely that the extra-reactivities are due to anti-virus activity in the sera because anti-virus activity could not be removed by such a procedure<sup>5,6</sup>.

In theory new antigenic expression can be due to: (1) direct alteration of the H-2 specificities on the cell surface as suggested by Doherty and Zinkernagel<sup>7</sup>; (2) introduction of new surface determinants coded for or induced by the virus genome; these determinants could cross react with and have similar properties as histocompatibility antigens<sup>7,8</sup>; (3) derepression of pre-existing genetic information for H-2 specificities of different haplotypes (see discussion by Martin<sup>9</sup> and Bodmer<sup>10</sup>).

We have no evidence for alteration of the normal H-2 specificities on the Meth A tumour cell since the cells from infected mice could absorb anti-H-2 antibody directed against the private specificities of the syngeneic strain (H-2D.4 and H-2K.31). The next possibility of direct viral action modifying the 'H-2 profile' or creating new 'H-2 like' determinants is an attractive one which we can neither prove nor disprove. An even more attractive possibility, for which we also have no proof, is derepression. Derepression could explain findings in the TL system in mice<sup>11</sup> and the Gm allotype system in man<sup>12</sup>, indicating that allelic differences may be the products of closely linked genes and the genetic polymorphism is exerted on the level of the control of whichever of the genes may become expressed<sup>10</sup>. With regard to our findings, it could be anticipated that the polymorphism breaks down when the virus interferes with the metabolism of the tumour cell.

It is not known whether other H-2-associated polymorphisms, like for instance lymphocyte-activating determinants (LADs) are also affected in this manner but some findings point in this direction: (1) leukaemic cells were able to stimulate syngeneic remission lymphocytes<sup>13,14</sup>; (2) during virus infection there was a transient stimulation in the mixed lymphocyte reaction between HLA identical lymphocytes (J. R. Salaman and H. Festenstein, unpublished); (3) influenza virus-infected lymphocytes stimulated syngeneic lymphocytes<sup>15</sup>; (4) the abnormally high stimulation of normal lymphocytes by human cell lines,

most likely infected with Epstein-Barr virus<sup>16</sup>. In this context particularly, it is interesting that we could not detect the emergence of Ia antigens on the infected tumour cells (Table 1).

Many questions are raised by these findings, particularly concerning the basis of surveillance against infections and tumours. The findings also highlight the role of the various components of the major histocompatibility system in alloimmune interactions<sup>17</sup>. If some tumours were to express one kind of H-2-like determinant rather than another, would this enable a tumour to grow better in some instances and less well in others, as recent reports<sup>8,18</sup> suggest? We have preliminary data indicating a suppressive effect of vaccinia virus on tumour growth and there is a recent report on a similar effect of vaccinia virus on human melanomas<sup>19</sup>. Relevant also in this context are experiments showing syngeneic graft rejection after infection by lymphomagenic virus<sup>20</sup>. Derepression of H-2 and possibly non-H-2 transplantation systems could be a normal surveillance mechanism analogous to the somewhat artificial situation of an alloimmune interaction. It also raises the question whether changes in immunogenicity induced chemically<sup>21</sup> or by irradiation (paper presented by J. McBurney and T. R. Munro, Second European Immunology Meeting, Amsterdam, 1975) could have similar mechanisms.

Our observations may point to the possibility of inducing rejection in one situation (tumours) and to provoking loss of immunogenicity in others (transplants). If this can be done, we may approach an understanding of "the pre-occupation of the immune system with histocompatibility antigens"<sup>1</sup> and the surveillance of transplants and tumours.

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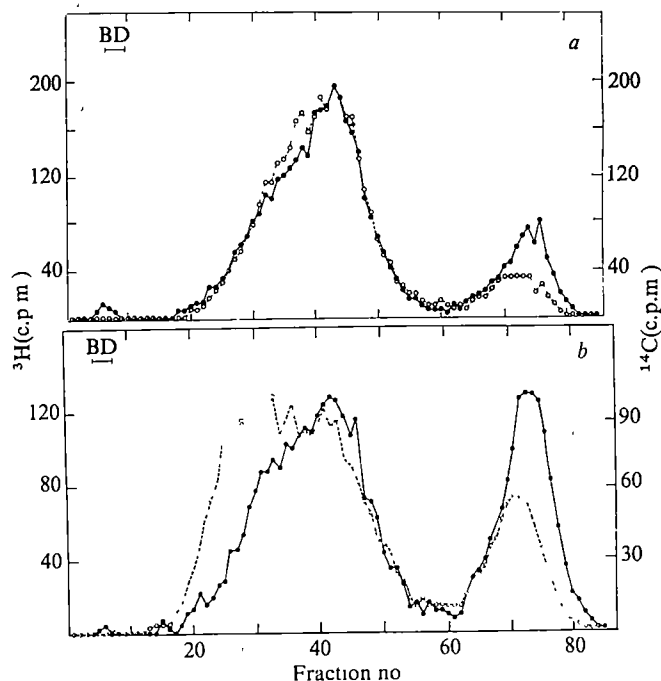
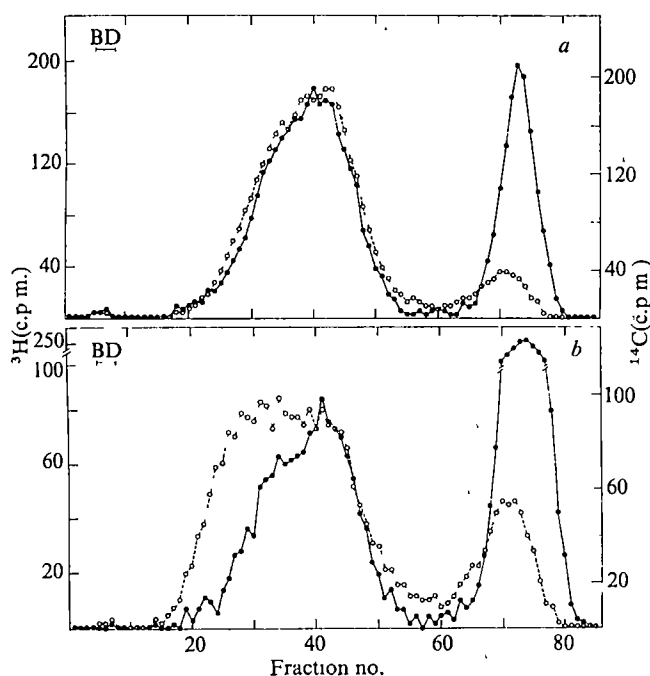
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## Surface membrane alterations in somatic cell hybrids of neuroblastoma and glioma cells

SOMATIC cell hybrids have been used to discern many properties of parental cells. Genes for neuronal properties can be activated in hybrids formed by fusion of mouse neuroblastoma and rat glioma cells (refs 1 and 2, and B. Hamprecht, T. Amano and M. Nirenberg, personal communication). These cell hybrids resemble the parent neuroblastoma cells in many of their properties as they can be induced to extend long neurites and possess electrically excitable membranes<sup>1</sup>. In addition the cell hybrids express other neuronal characteristics that are less pronounced in the parental cells, such as choline acetyltransferase<sup>1</sup>, and morphine receptors<sup>2</sup>. Many of the above properties suggest alterations in the surface membrane. We have shown previously that a particular group of glycopeptides is more prominently expressed on the cell surface in clones of mouse neuroblastoma which have the ability to undergo morphological differentiation than in clones that are unable to extend neurites<sup>3</sup>. In the present report, we show that this group of glycopeptides is abundant in the surface membranes of the hybrid cells.

The parent cell lines were: N18TG-2, a mouse neuroblastoma clonal line resistant to 6-thioguanine<sup>4</sup> and C6BU-1, a 5-bromodeoxyuridine-resistant clone of rat glioma<sup>1</sup>.

**Fig. 1** Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma clone N18TG-2 and hybrid NG108-15 cells grown in the presence of L-<sup>14</sup>C- or <sup>3</sup>H-fucose, respectively. Trypsinates were combined, digested with Pronase and chromatographed as described<sup>3</sup>. Sephadex G-50 profile of Trypsinate A (loosely-associated glycopeptides) (a) and Trypsinate B (tightly-associated glycopeptides) (b) from N18TG-2 (○) and NG108-15 (●) cells. BD represents fractions in which Blue Dextran 2000 was eluted. Phenol red eluted at fraction 105.



**Fig. 2** Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma N18TG-2 and hybrid NG108-5 cells grown in the presence of L-<sup>14</sup>C- or <sup>3</sup>H-fucose, respectively. Trypsinate A (a) and Trypsinate B (b) from N18TG-2 (○) and NG108-5 (●) cells. Details are described in Fig. 1.

Hybrids of these parental lines, NG108-5 and NG108-15 were obtained by Sendai virus-induced fusion of the neuroblastoma and glioma clones (B. Hamprecht, T. Amano and M. Nirenberg, personal communication). All these cells were obtained from Dr M. Nirenberg, NIH. The modal chromosome numbers for C6-B4-1 and N18TG-2 are 39 and 83 respectively, whereas the hybrids have chromosome numbers higher than the sum of the parental cells. These and other characteristics of the hybrids have been reported<sup>5,6</sup>. The cells were grown to confluency in Dulbecco's modified Eagle's medium (DMEM) with 10% foetal calf serum, at which time the glycoproteins were made radioactive by incubating the cells for 2 d in medium containing L-<sup>3</sup>H- or <sup>14</sup>C-fucose (4.8 Ci mmol<sup>-1</sup> and 57 mCi mmol<sup>-1</sup>, respectively, Amersham). These procedures as well as those for collecting cells have been described<sup>3</sup>. The membrane glycopeptides were removed from the cell surface by sequential trypsinisation since different groups of glycopeptides are distinguished by this procedure<sup>3</sup>. One group is preferentially removed by the first trypsinisation step (Trypsinate A) suggesting that these glycopeptides are more accessible or loosely associated with the membrane<sup>7</sup>. Trypsinate B, which is removed with the second trypsin treatment, contains the more tightly-associated glycopeptides. All radioactive trypsinates to be compared were combined, digested exhaustively with Pronase (Calbiochem) and chromatographed on a column of Sephadex G-50 (ref. 3). Choline acetyltransferase (CAT) and acetylcholinesterase (AChE) were assayed according to Blume *et al.*<sup>8</sup>. Duplicate extracts at two concentrations of cells in the confluent state were used. The formation of acetylcholine by CAT was verified using electric eel AChE<sup>9</sup>. Specific AChE was determined by including 10<sup>-5</sup> M BW-284C51 dibromide (Wellcome) in the assay mixture.

The chromatographic profiles of the loosely-associated glycopeptides (Trypsinate A) from the surface of the parent neuroblastoma and hybrid cells were found to be similar (Figs 1a and 2a), and slightly different from that of the parent glioma cells (Fig. 3a). On the other hand, the pattern of the more tightly-associated glycopeptides

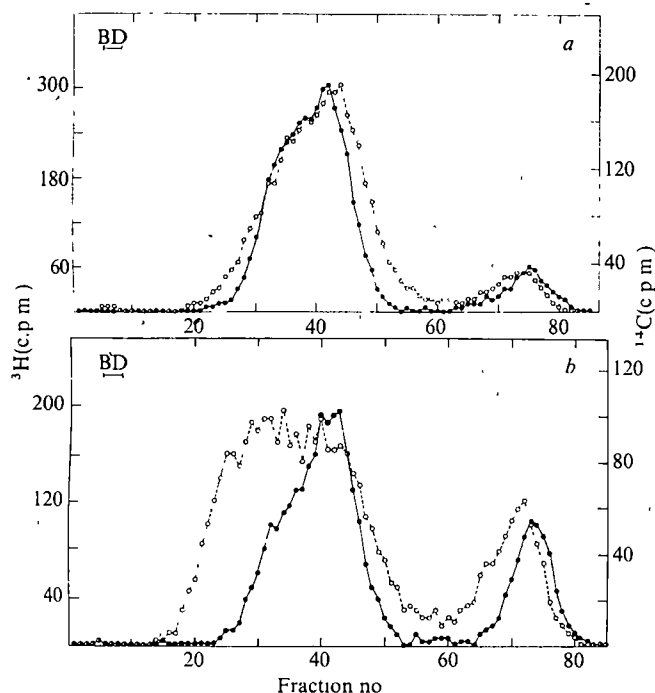
**Table 1** L-<sup>3</sup>H-fucose incorporation into parental and hybrid cells

Clonal lines	L- <sup>3</sup> H-fucose incorporation		Trypsinate	
	c.p.m. per 10 <sup>6</sup> cells	c.p.m. per µg protein	A	B
Parents				
Mouse neuroblastoma				
N18TG-2	2,150	63	4	3
Rat glioma C6BU-1	235	15	8	4
Hybrids				
NG108-5	13,134	154	7	2
NG108-15	4,235	74	7	4

Confluent cultures, in 60-mm dishes, were incubated in 7 ml medium containing L-<sup>3</sup>H-fucose for an additional 51 h (477,500 c.p.m. per ml medium). Cells were washed four times with cold 0.16 M NaCl and removed from the monolayer with 1 ml 0.25% trypsin solution (3 min at room temperature). To the cell suspension, 1 ml medium containing 10% foetal calf serum was added and aliquots removed for radioactive counting. Values for the trypsinates were obtained from another set of radioactively labelled cultures. Sequential trypsinisation was carried out as described previously<sup>3</sup>.

(Trypsinate B) from the parent neuroblastoma cells differed markedly from those of the hybrid and glioma cells (Figs 1b, 2b and 3b). The chromatographic profiles of the tightly-associated glycopeptides from the parent neuroblastoma cells may be divided into early eluting groups (Fig. 1b, fractions 15–30), more slowly eluting groups (Fig. 1b, fractions 31–50) and dialysable material (fractions 65–80). The latter includes free fucose, and the significance of this material requires further investigation. The early eluting glycopeptides were missing from the material derived from the surface of the hybrid cells (Fig. 1b and 2b) and the parent glioma cells (Fig. 3b). In contrast, the more slowly eluting glycopeptides (fractions 31–50) were similar in all of the cell lines examined. This latter group (fractions 31–50) was also prominent in the neuroblastoma clones which are capable of morphological differentiation and less pronounced in the surface glycopeptides from axon-minus cells<sup>3</sup> or these cells in logarithmic phase of growth (M.C.G., Y.K. and U.Z.L., unpublished).

**Fig. 3** Chromatography on Sephadex G-50 of Pronase-digested trypsinates from mouse neuroblastoma N18TG-2 and rat glioma C6BU-1 cells grown in the presence of L-<sup>14</sup>C- or <sup>3</sup>H-fucose, respectively. Trypsinate A (a) and Trypsinate B (b) from N18TG-2 (○) and C6BU-1 (●) cells. See Fig. 1.



In accordance with previous results (B. Hamprecht, T. Amano and M. Nirenberg, personal communication), high levels of CAT were found in the hybrid cell lines, whereas the activity of this enzyme in both parent cells was low (143,160, 58 and 3.8 pmol per mg protein per min for NG108-5, NG108-15, N18TG-2 and C6BU-1, respectively). The expression of this enzyme was also reported to be activated in a hybrid clone of mouse neuroblastoma NA and human fibroblasts<sup>10</sup>. In addition, AChE activity was expressed in the two hybrids and the parent neuroblastoma cells (14,700, 7,480 and 7,980 pmol per mg protein per min). The parent glioma cells had less activity (82 pmol per mg protein per min).

Less radioactive L-fucose was incorporated into the glioma cells than into either the hybrid or neuroblastoma cells, either expressed as per cell or per mg cell protein. The latter takes into account the size difference of the cell lines (Table 1). Since the percentages of the total incorporated fucose which were removed by the trypsinisations were similar, the fucose-containing glycoproteins may be deficient in the membranes of the glioma cells. The cells were grown in the presence of radioactive fucose for 51 h, so the amounts probably do not reflect increased turnover of the membrane glycoproteins. These results suggest that in this phenotypic expression of the fucose-containing glycoproteins the traits more characteristic of the neuroblastoma cells continue to be preserved or even activated in the hybrids. Closer scrutiny of the fucose-containing glycopeptides using chromatographic profiles (Figs 1b and 2b), however, showed only a partial preservation in the hybrids of the phenotypic expression of the neuroblastoma cells. More detailed analyses of the glycoproteins will be required to determine if the glioma phenotype is preserved at all.

The hybrid cell lines were shown to undergo biochemical, morphological and electrical differentiation (B. Hamprecht, T. Amano and M. Nirenberg, personal communication). For this reason, we anticipate that analysis of the membrane glycopeptides from a variety of hybrids will reveal which glycopeptides are associated with the acquisition of these properties. We conclude from the present studies that the glycopeptides which elute early from the Sephadex G-50 column may not be connected with the phenotypic expression of the differentiated state in neuroblastoma cells, since they are missing from the surface of the hybrid cells. It follows that the slowly eluting group of glycopeptides may be associated with the ability to neuroblastoma cells to differentiate. This group consists of several glycopeptides<sup>7</sup>. Since the glioma cells also show a corresponding group of slowly eluting glycopeptides it may be possible with further chemical studies to distinguish between those which the cells may have in common and those which correlate with differentiation. The fact that hybrid cells do not possess the early eluting glycopeptides substantiates our earlier finding<sup>3</sup> that the slowly eluting glycopeptides are characteristic of the differentiated state of neuroblastoma cells.

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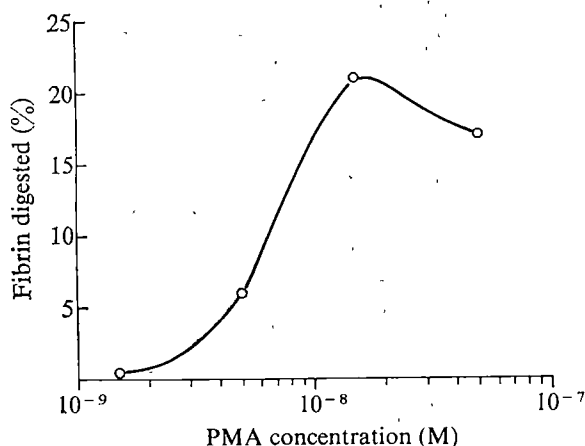


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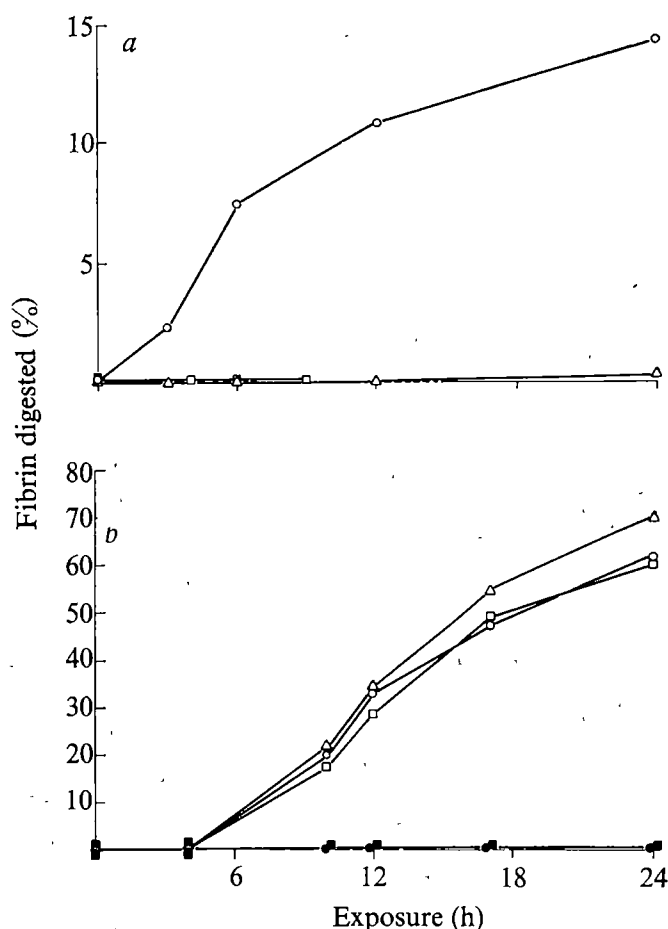
## Tumour promotor induces plasminogen activator

INFECTION of chick embryo fibroblasts with Rous sarcoma virus (RSV) induces a cell-specified plasminogen activator<sup>1</sup>. Induction occurs with transforming viruses but not with lytic viruses or with oncornaviruses which are not themselves transforming<sup>2</sup>. Similarly, many mammalian cell lines and embryo cultures transformed with either viruses or chemical carcinogens may be high producers of plasminogen activator in contrast to their untransformed counterparts<sup>3-6</sup>. A correlation has been demonstrated between production of plasminogen activator and various features of the transformed phenotype, such as cell locomotion, morphology and loss of anchorage-dependent growth<sup>4,7</sup>. Several established cell lines which are not highly tumorigenic or transformed by the usual criteria are, however, active producers of plasminogen activator; there are examples also of transformed or tumorigenic cell lines which do not produce significant levels of plasminogen activator<sup>3,8-10</sup>.

We have been interested in the regulation of the expression of plasminogen activator in the expectation that an understanding of the mechanism of its control and an ability to alter its levels with exogenous agents would provide insights into its physiological significance in the



**Fig. 1** A dose study of PMA in CEF cells. Subconfluent plates of CEF (approximately  $5 \times 10^6$  cells per 9-cm dish) were re-fed growth medium containing PMA at the indicated concentrations. After 24 h cells were washed twice with phosphate-buffered saline and collected by scraping into 2 ml of swelling buffer (10 mM Tris, 10 mM NaCl, pH 8.0) without detergents. Lysates were diluted 1:10 into assay buffer (100 mM Tris, 10 mM NaCl, 3 mM  $\text{NaN}_3$ , pH 8.0) and assayed in triplicate as described by Unkeless *et al.*<sup>1</sup> on fibrin-coated plates containing 40,000-70,000 c.p.m. of  $^{125}\text{I}$ -fibrin ( $10 \mu\text{g cm}^{-2}$ ) in the presence of 4  $\mu\text{g per ml}$  human plasminogen previously purified by lysine-Sepharose affinity chromatography<sup>13</sup>. Aliquots were taken after 3 h and the solubilised fibrin determined by scintillation counting (Nuclear Chicago) in Aquasol (New England Nuclear Corp.) using the  $^3\text{H}$  channel. Percentage fibrin digested was determined from the ratio of the counts released in the presence of sample (adjusted for fibrin released in the presence of plasminogen but in the absence of sample) to the total counts released by trypsin.



**Fig. 2** Time course of induction. *a*, Cell lysate assay. Agents were added to replicate subconfluent plates of CEF at zero time and at the subsequent times indicated in the figure cells were collected and lysates assayed as described in Fig. 1.  $\Delta$ , Untreated CEF;  $\circ$ , CEF treated with  $5 \times 10^{-8}$  M PMA;  $\square$ , CEF treated with  $5 \times 10^{-8}$  M PMA and  $1 \mu\text{g ml}^{-1}$  actinomycin D. *b*, Intact cell assay. Assays were carried out according to Unkeless *et al.*<sup>2</sup>. CEF ( $2 \times 10^6$ ) were plated in triplicate on to 6-cm plates coated with 50,000 c.p.m.  $^{125}\text{I}$ -fibrin ( $10 \mu\text{g cm}^{-2}$ ). The medium was Dulbecco's with 10% foetal calf serum. Six hours after plating (zero time), various agents were added to the plates. Aliquots were taken at the times indicated in the figure and percentage fibrin digested determined as described in the cell lysate experiments.  $\bullet$ , Untreated CEF;  $\square$ , CEF treated with  $1.5 \times 10^{-8}$  M PMA;  $\circ$ , CEF treated with  $1.5 \times 10^{-8}$  M PMA and  $10^{-6}$  M phorbol;  $\blacksquare$ , CEF treated with  $10^{-6}$  M phorbol;  $\Delta$ , CEF treated with  $5 \times 10^{-8}$  M PMA.

normal and malignant state. We reported that glucocorticoids inhibit expression of plasminogen activator in certain cell lines<sup>10</sup>. Troll *et al.* found that a phorbol ester, phorbol-12-myristate-13-acetate (PMA), a component of croton oil which causes epidermal inflammation, hyperplasia and tumour promotion in treated skin<sup>11</sup>, also induces increased trypsin-like proteolytic activity in treated mouse skin<sup>12</sup>. In view of these findings, we tested the effects of PMA on plasminogen activator levels in cultured cells, and report here that PMA is a potent inducer of plasminogen activator in chick embryo fibroblasts and HeLa cells.

Plasminogen activator was assayed by published methods<sup>1,2</sup> (see legends to Figs and Table for details). The assay is based on quantification of the solubilisation of  $^{125}\text{I}$ -labelled fibrin due to proteolytic digestion in the presence of plasminogen and sample. The sample may be either intact cells, plated directly on to a fibrin-coated dish, or a lysate of cells. In our studies, omission of plasminogen from the assay of PMA-induced cells or cell lysates reduced fibrinolysis by more than 95%, indicating that the fibrinolysis was plasminogen dependent. This justifies our referring throughout to the induction of a plasminogen activator.

**Table 1** Maximum-fold increase in cell-associated plasminogen activator over endogenous levels after exposure to PMA

Cell	Origin	Time in culture	Maximum-fold increase in plasminogen activator
CEF	Chick embryo fibroblast	1-3 weeks	10
HeLa <sup>20</sup>	Human cervical carcinoma	20 yr	10
HEC	Hamster embryo cells	1-3 weeks	3
REC	Rat embryo cells	1-3 weeks	2
HTC <sup>21</sup>	Rat hepatoma	10 yr	2
L <sup>20</sup>	Mouse fibroblast	20 yr	1

Exposure was for 24 h and ranged from 10 to 1,000 ng ml<sup>-1</sup>. Cells were grown as monolayer cultures in either Dulbecco's modified Eagle's minimal essential medium (DMEM) with 5% foetal calf serum (FCS) (L cells), DMEM+10% FCS (CEF, HEC, REC, HeLa) or Ham's F12 Medium + 10% FCS (HTC). Embryo cultures were prepared by standard procedures<sup>22,23</sup> from 9-12-d chick embryos, midterm pregnant rats or late term pregnant hamsters. Cell lysates were prepared and assayed as described in the legend to Fig. 1.

The dose-response curve of chick embryo fibroblasts (CEF) to PMA is shown in Fig. 1. Induction occurred at a concentration as low as  $5 \times 10^{-9}$  M PMA ( $3 \text{ ng ml}^{-1}$ ). Maximal induction was observed at  $1.5 \times 10^{-8}$  M. The time course of induction of plasminogen activator in CEF as assayed in cell lysates and intact cells is shown in Fig. 2a and b. Increased fibrinolytic activity in lysates (cell-associated plasminogen activator) was observed as early as 3 h after exposure to PMA (Fig. 2a). The longer delay in the appearance of extracellular fibrinolytic activity assayed with intact cells (Fig. 2b) reflects the lower sensitivity of this assay, since in Fig. 2b foetal calf serum was used as a source of plasminogen. When intact cells were assayed using foetal calf plasminogen, purified by lysine-Sepharose affinity chromatography<sup>13</sup>, rather than serum, induction was detected after 3 h (data not shown). Phorbol itself, which is inactive as a tumour-promoting agent<sup>11</sup>, neither induced nor blocked induction (Fig. 2b). Actinomycin D completely blocked the response to PMA (Fig. 2a), suggesting that PMA induction requires RNA synthesis.

The induced levels of plasminogen activator gradually returned to control values within 12 h of removal of PMA. Curiously, the deinduction was also blocked by actinomycin D. In this regard, deinduction resembles the glucocorticoid-induced repression of plasminogen activator expression in rat hepatoma cells, which is also blocked by actinomycin D (ref. 10).

PMA is not toxic to early passage CEF at  $5 \times 10^{-8}$  M as judged by growth rate during a 4-d exposure; however, it did inhibit growth of late (eighth) passage CEF. A growth inhibitory effect of PMA on late passage CEF has also been reported by Diamond *et al.*<sup>14</sup>. In spite of the different effects on growth at early and late passage, the kinetics of induction of plasminogen activator were found to be the same at both passage times.

Several cell types were screened for response to PMA (Table 1). PMA produced approximately a tenfold elevation of plasminogen activator in HeLa and CEF. A two- to threefold increase was seen with hamster embryo cells, rat embryo cells and a rat hepatoma cell line, and no induction was found with the mouse L cell line. Unresponsive cell types may prove useful in an analysis of the mechanism of the PMA response.

Phorbol esters, in addition to causing epidermal hyperplasia, inflammation and tumour promotion, have various other biological effects. These include alterations in cell morphology, growth properties and increased <sup>3</sup>H-choline incorporation in cultured cells<sup>14-16</sup>; mitogenesis in mouse 3T3 cells and lymphocytes<sup>17</sup>; a rapid but short lived increase in cyclic GMP in lymphocytes and mouse 3T3 cells<sup>17</sup>; decreased cyclic AMP levels in mouse skin<sup>18</sup>; and enhancement of cell transformation by chemical carcinogens in cell culture<sup>19</sup>. Our discovery that extremely low concentrations of PMA induce a marked increase in plasminogen activator in such diverse systems as CEF and

HeLa cells, together with the previously described *in vitro* effects of this compound, suggest that PMA induces a constellation of changes reminiscent of the phenotype of cells transformed by oncogenic viruses or chemical carcinogens, a major difference being that the effects of PMA are readily reversed when the agent is removed. An analysis of the mechanism of action of PMA in cell culture may, therefore, shed light on the general problem of malignant transformation.

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## Evidence for inhibition of platelet activation in blood by a drug effect on erythrocytes

ADP released from erythrocytes causes adhesion and aggregation of platelets<sup>1</sup>. This can presumably happen *in vivo*

when circulating red cells undergo haemolysis in pathological conditions<sup>2,3</sup>, even though ADP is hydrolysed by enzymes present in blood<sup>4-7</sup>. More important pathologically, as a factor in thrombogenesis, is the possibility that ADP is also released from red cells which are structurally and metabolically normal. ADP might leak from circulating cells undergoing reversible deformations<sup>8</sup> which are much increased by abnormal conditions of flow, for example in vessels narrowed by atherosclerosis.

Experimentally, the adhesiveness of platelets to glass is increased by the presence of erythrocytes without evidence of haemolysis; and the increase is abolished by apyrase which catalyses the dephosphorylation of ADP<sup>9</sup>. When heparinised blood from anaesthetised pigs is made to flow through extra-corporeal shunts, deposits of platelets form at branch points but nowhere else<sup>10</sup>; and no deposits form with platelet-rich plasma, indicating that haemodynamic effects on red cells suffice to increase platelet adhesiveness.

The resistance of erythrocytes to hypotonic haemolysis is increased by low concentrations of various drugs, including chlorpromazine and other phenothiazines<sup>11</sup>. The mechanism by which these substances increase the resistance of red cells may alter their membrane so as to diminish or prevent the leakage of ADP; and if so, such an effect should show itself as a diminution of the reactivity of platelets in flowing blood. We have therefore determined the effect of chlorpromazine on the 'bleeding time' from a small hole in an artificial vessel, the extravasation being terminated, as *in vivo*, by a plug of platelets. The proposition that any diminution in platelet reactivity resulted from an effect of chlorpromazine on the red cells required that the bleeding time was prolonged by concentrations below those at which the drug inhibits the release reaction of platelets<sup>12</sup>.

Human blood was obtained from the antecubital veins of apparently healthy volunteers who had not taken aspirin or any similar drug in the preceding week. The blood was mixed with trisodium citrate (final concentration 0.38%) as anticoagulant. The concentration of platelets varied from  $1.36 \times 10^8$  to  $2.40 \times 10^8$  ml<sup>-1</sup>. Blood was centrifuged at 280g for 15 min for preparing plasma containing  $2.40 \times 10^8$ – $3.82 \times 10^8$  platelets ml<sup>-1</sup>.

For each experiment, whole blood or platelet-rich plasma was pumped continuously from a reservoir through polyethylene tubing about 30 cm long and 0.3 mm internal diameter; the flow rate was 0.16 ml min<sup>-1</sup>. The tube crossed a glass plate under a microscope (Leitz Laborlux) where it was continuously bathed in a stream of Tyrode's solution at 37.5 °C. A small cut was made through about one-third of the circumference of the tube and the duration of 'bleeding' into the bathing solution

was determined up to a maximum time of 600 s. Results are presented as median as well as mean ( $\pm$ s.e.m.) bleeding times in Table 1.

For blood alone the median bleeding time was 130 s and for platelet-rich plasma 600 s. Thus, although the concentration of platelets was always higher in plasma than in blood, the bleeding time was much shorter with blood than with plasma; presumably the difference was due to some influences of the red cells.

Chlorpromazine (Hibernal; Leo AB Halsingborg, Sweden) was added to blood in the reservoir and the median bleeding time was more than doubled by the drug at  $1.6 \times 10^{-6}$  M, which is about 30 times less than the concentration required for halving the release reaction *in vitro*. Higher concentrations increased the bleeding time further, so that the 10-min limit was reached with about  $1 \times 10^{-5}$  M chlorpromazine.

$\beta$ -Diethylaminoethyl-diphenylpropylacetate (Proadifen; SKF 525A, Smith, Kline and French Ltd, Welwyn Garden City, England) also increases the resistance of erythrocyte membranes<sup>13</sup> and at  $1 \times 10^{-6}$  M increased the median bleeding time almost threefold.

The median bleeding time was also prolonged increasingly by the enzyme apyrase (Sigma) in concentrations of 0.5–50  $\mu$ g ml<sup>-1</sup>; but less at 500  $\mu$ g ml<sup>-1</sup>, possibly because of a nonspecific effect of so much added protein. The increase in bleeding time caused by apyrase is most simply accounted for by an accelerated breakdown of free ADP, preventing its activating effect on platelets.

The observations are compatible with our proposition that a drug such as chlorpromazine, which increases the resistance of red cells, can decrease the leakage of ADP from them in conditions in which this shows itself as a diminution in the activation of platelets in the blood. The hypothesis is thus that the activation of platelets by red cells depends on a chemical mechanism produced by released ADP. In flowing blood, red cells also affect platelets through a physical mechanism, by increasing the diffusivity of platelets by up to two orders of magnitude (see ref. 14). Presumably this greatly increases the frequency with which circulating platelets collide with vessel walls and such a physical effect is, indeed, required to account for the rate at which platelet thrombi are observed to grow when produced experimentally in living blood vessels<sup>15-17</sup>. The absence of platelet aggregation in plasma which is stirred at high rates<sup>18</sup>, however, indicates that the collisions on which the adherence of platelets to vessel wall and to each other depend do not by themselves make the platelets adhesive. That depends on their chemical activation by ADP.

**Table 1** Effect of chlorpromazine, SKF 525A or apyrase on the median and mean bleeding times from a small cut in polythene tubes (for method see text)

		see text					
	Addition	Concentration	Number of determinations	Bleeding time (s)		Bleeding time greater than 10 min (%)	
				Median	Mean ( $\pm$ s.e.m.)		
Blood	None (Controls)		70	130	208	19.7	7
	Chlorpromazine	(M)					
		$1.6 \times 10^{-6}$	15	280	271	40.3	7
		$3.2 \times 10^{-6}$	15	390	396	53.3	40
		$8.0 \times 10^{-6}$	15	560	437	47.3	40
		$1.6 \times 10^{-5}$	15	600	(509)	41.4)	73
	SKF 525A	$1.0 \times 10^{-6}$	10	300	340	64.4	10
		$1.0 \times 10^{-5}$	10	540	400	70.4	40
		$1.0 \times 10^{-4}$	11	600	(551)	31.6)	82
	Apyrase	( $\mu$ g ml <sup>-1</sup> )					
		0.5	10	360	349	74.3	40
		5.0	15	430	390	48.3	33
		50	14	600	(546)	29.8)	57
		500	15	170	277	58.6	27
Platelet-rich plasma	None		28	600			96

Other possible explanations of our observations have to be considered. In altering the deformability of erythrocytes<sup>19</sup>, chlorpromazine might affect their flow properties such as to diminish the probability of platelet collisions. Any such effect is unlikely to make a measureable difference in our experiments; nor does it overcome the requirement that the platelets have to be activated before aggregating as an haemostatic plug. Another possibility is that chlorpromazine interferes with the aggregation of platelets after their activation by ADP, for example by competing with calcium<sup>20</sup> which is essential for aggregation. If this were the explanation, however, primary aggregation by added ADP should be inhibited by chlorpromazine in platelet-rich plasma, and this is not so<sup>12</sup>. Furthermore, the concentrations of chlorpromazine which prolong the bleeding time are much lower than the calcium concentration required for platelet aggregation, even in the presence of citrate as anticoagulant.

Direct flow-mechanical activation of platelets has been demonstrated experimentally but in very different conditions<sup>21</sup>. Haemodynamic disturbances produce platelet thrombi in the blood channels of artificial organs such as kidneys or oxygenators, whereby their function is often terminated prematurely<sup>22</sup>. The conclusion that the red cells are primarily responsible for these thrombi accords with the fact that thrombus formation is similar in channels made of various materials as long as the geometry is the same. Our results, therefore, suggest a new way of prolonging the function of such artificial organs through the addition of an agent capable of inhibiting the release of ADP (and ATP) from erythrocytes. Such an agent should, of course, have as few other effects as possible, so that chlorpromazine, although effective, would not be the choice.

Our observations similarly suggest a new approach to the prophylaxis of those thrombotic diseases in which thrombus formation depends on platelets, as in a proportion of acute coronary thromboses. This approach would require the demonstration that the incidence of such a disease is diminished by drugs which, in clinically acceptable blood concentrations, do not inhibit platelet function directly and which inhibit the release of ADP experimentally from non-haemolysing erythrocytes during rheological stress. Our hypothesis may also explain the effect of dipyridamole and sulphapyrazone in preventing increased utilisation of circulating platelets in potentially thrombogenic conditions<sup>23</sup>. This cannot easily be accounted for by any direct action on platelets by either drug at its clinically effective concentration; instead, these drugs may act on the red cells to diminish their activation of platelets.

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## Inhibition of plasmin-mediated fibrinolysis by vitamin E

BIOLOGICAL effects attributed to vitamin E include the maintenance of cell membrane integrity, inhibition of enzyme-dependent lipid peroxidation, participation in oxidative phosphorylation, and a general, non-enzymatic antioxidant effect<sup>1</sup>. In spite of these observations, the physiological function of the vitamin remains obscure. Natural deficiency in man has been invoked in the pathogenesis of several disorders, with convincing evidence limited to a specific type of haemolytic anaemia<sup>2</sup>. Therapeutically, vitamin E has its advocates in treatment of peripheral vascular and thromboembolic disease, although documentation for its efficacy is meagre<sup>3</sup>. Although it is considered non-toxic<sup>4</sup>, little is known about adverse effects of excessive dietary vitamin E.

Plasminogen is the plasma proenzyme which, on conversion to its active form, plasmin, is considered responsible for lysis of fibrin deposits resulting from physiological or pathological activation of the coagulation cascade<sup>5</sup>. In spite of interest in vitamin E as therapy for vascular disease, and the importance of coagulation and fibrinolysis in intravascular homeostasis<sup>6</sup>, information on its effects on these processes is limited. An anti-thrombin effect has been claimed<sup>6</sup>, but not confirmed<sup>7</sup>. Prolongation of plasma clotting time has been reported with vitamin E therapy, together with decrease in the expected increment in blood fibrinolytic activity which follows venous occlusion<sup>7</sup>. We report a direct effect of vitamin E on the fibrinolytic system, namely, *in vitro* inhibition of plasmin-mediated fibrinolysis at physiological concentrations of the vitamin and enzyme.

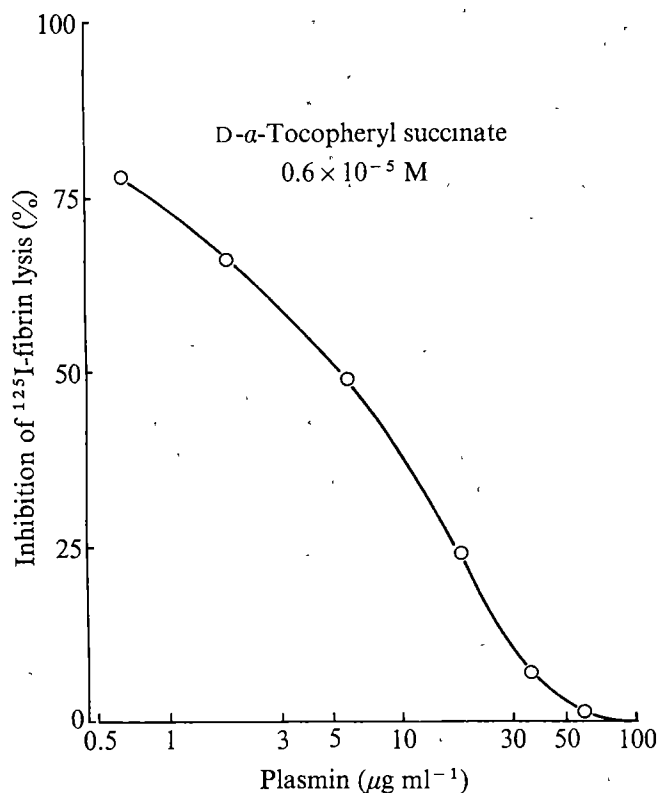
Fibrinolysis was assayed by a sensitive solid phase <sup>125</sup>I-fibrin tube method. This technique, described in detail elsewhere<sup>8</sup>, is based on release of radioactive degradation products from <sup>125</sup>I-fibrin (human), originally absorbed to polystyrene tubes as fibrinogen, and converted to non-cross linked fibrin by thrombin. Plasminogen was isolated from normal human plasma by affinity chromatography on lysine-Sepharose<sup>9</sup>, and subsequent stepwise<sup>9</sup> or gradient elution<sup>10</sup> with ε-aminocaproic acid (EACA). Plasmin was

**Table 1** Effect of vitamin E on lysis of <sup>125</sup>I-fibrin by plasmin and other proteolytic enzymes

	<sup>125</sup> I-fibrin lysed (c.p.m.)		
	- Vitamin E	+ Vitamin E	Δ
Plasmin	9,968	1,634	-84%
Collagenase (Clostridiopeptidase A, Worthington)	18,941	17,870	-6%
Chymotrypsin (α-chymotrypsin, bovine pancreas, Worthington)	36,070	39,539	+10%
Trypsin (bovine pancreas, Worthington)	38,380	44,363	+16%
Pronase (bacterial protease, type VI, Sigma)	51,001	67,864	+33%

Enzyme (5 μg ml<sup>-1</sup>) and vitamin E (1.7 × 10<sup>-5</sup> M) were incubated in <sup>125</sup>I-fibrin coated assay tubes for 30 min at 37 °C (final volume, 0.2 ml in Tris-NaCl buffer). Results are means of duplicate assays. Assay tubes contained 145,000 c.p.m. of <sup>125</sup>I-fibrin (90,000 c.p.m. per μg fibrin).





**Fig. 1** Effect of vitamin E on lysis of <sup>125</sup>I-fibrin by human plasmin. Plasmin, at varying concentrations, was incubated with or without vitamin E ( $0.6 \times 10^{-5}$  M) at  $37^\circ\text{C}$  for 30 min (final volume, 0.2 ml) in assay tubes coated with <sup>125</sup>I-fibrin (140,000 c.p.m. per tube, 90,000 c.p.m. per μg fibrin). Lysis in presence of vitamin E is expressed as percentage of lysis by plasmin alone. Nonspecific release of radioactivity with buffer alone (289 c.p.m.) was subtracted from experimental values. Release with vitamin E alone ( $2.5 \times 10^{-5}$  M), 270 c.p.m. Experimental values (means of duplicate assays) at representative plasmin concentrations (0.6, 6 and 60 μg ml<sup>-1</sup>, values with vitamin E in parentheses): 364 (84), 6,504 (3,205) and 12,684 (11,870) c.p.m., respectively.

in the form of spontaneously activated plasminogen<sup>8</sup>, as judged by absence of generation of additional activity by appropriate streptokinase or urokinase treatment. Plasminogen and plasmin were quantitated by absorbancy at 280 nm (ref. 11). Vitamin E, in the form of D-α-tocopheryl succinate (type VI, Sigma) was suspended in Tris-NaCl buffer (0.015 M Tris, 0.15 M NaCl, pH 7.4) at a concentration of 1 mg ml<sup>-1</sup>, stirred for 15 min at room temperature, and filtered through Whatman No. 1 paper. Vitamin E content of the filtrate was quantitated by absorbancy at 292 nm (ref. 12), and colorimetrically by the tocopherol red reaction<sup>13</sup>, after preliminary alkaline saponification to yield the free phenol. Concentrations of vitamin E obtained in this way were  $3 \times 10^{-5}$  to  $4 \times 10^{-5}$  M.

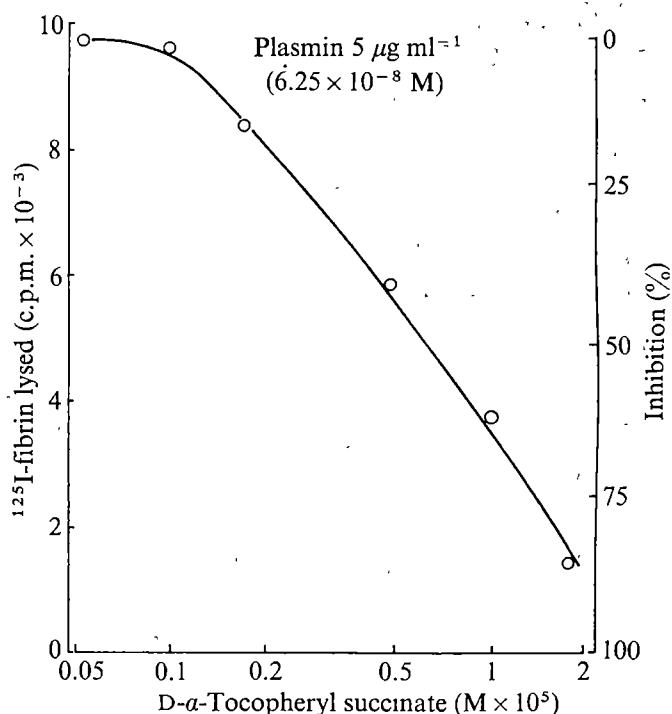
Vitamin E ( $0.6 \times 10^{-5}$  M) produced 50% inhibition of fibrinolysis at a plasmin concentration of  $5 \mu\text{g ml}^{-1}$ , or  $0.6 \times 10^{-7}$  M (molecular weight of plasmin, 81,000 (ref. 14), an inhibitor-enzyme molar ratio of 100:1 (Fig. 1). Examination of the effects of varying concentrations of vitamin E (plasmin,  $5 \mu\text{g ml}^{-1}$ ) was confirmatory (Fig. 2), with 50% inhibition at a vitamin E concentration of  $0.5 \times 10^{-5}$  to  $0.6 \times 10^{-5}$  M. Sodium succinate ( $5 \times 10^{-5}$  M) or filtered buffer had no effect. Since vitamin E functions as an antioxidant<sup>1</sup>, L-cysteine hydrochloride was tested. No effect on fibrinolysis was observed at concentrations from  $10^{-7}$  to  $10^{-3}$  M. Pretreatment of assay tubes with vitamin E ( $2 \times 10^{-5}$  M,  $37^\circ\text{C}$ , 30 min) had no effect on subsequent <sup>125</sup>I-fibrin lysis. The effect of vitamin E on fibrinolysis by plasmin generated by streptokinase or urokinase activation of purified plasminogen<sup>9</sup> was identical to that with spontaneously activated plasmin. The inhibition was also

demonstrable by the fibrin plate method<sup>17</sup>, in which non-radioactive fibrin is used as substrate (Fig. 3). Finally, addition of vitamin E to urokinase-stimulated normal plasma, at concentrations producing 50% inhibition of purified plasmin, resulted in 25% inhibition of <sup>125</sup>I-fibrin lysis.

Some observed effects of vitamin E esters, particularly the phosphate, which inhibits trypsin and papain<sup>15</sup>, have been attributed to their anionic nature rather than to tocopheryl group<sup>1</sup>, and may be mimicked (in the case of inhibition of hyaluronidase) by heparin and sodium dodecyl sulphate<sup>16</sup>. Neither of these agents, tested at  $10^{-5}$  M, inhibited lysis of <sup>125</sup>I-fibrin by plasmin. Vitamin E did not inhibit lysis by four other proteolytic enzymes known to digest this substrate<sup>5,8</sup> (Table 1), at similar molar concentrations of the enzymes (maximal estimated concentrations,  $0.5 \times 10^{-7}$  M for collagenase to  $3.3 \times 10^{-7}$  M for trypsin). Additional evidence for the absence of an anionic effect has been provided by affinity chromatography experiments. D-α-tocopheryl succinate was coupled to aminohexyl Sepharose 4B (AH Sepharose, Pharmacia) by the carbodiimide reaction. With a column (1 ml bed volume) of this conjugate, in which the succinyl carboxyl group is blocked, application of purified plasmin (260 μg) resulted in binding of 80%, and subsequent elution of 60% of the bound plasmin by buffer containing D-α-tocopheryl succinate. Finally, inhibition of fibrinolysis by plasmin does not appear to be due to the ester form of the vitamin, since similar inhibition was produced by the free phenol. Pure D-α-tocopherol (Eastman Kodak), dissolved in ethanol because of its insolubility, was added to and incubated with plasmin ( $5 \mu\text{g ml}^{-1}$ , 30 min at  $37^\circ\text{C}$ ). Fifty per cent inhibition of plasmin lysis of <sup>125</sup>I-fibrin occurred at a free phenol concentration of  $1.6 \times 10^{-5}$  M, which compares with similar inhibition by the succinate ester at  $0.6 \times 10^{-5}$  M (Fig. 2). Lysis by plasmin plus ethanol (1.5% by volume) was 3,641 c.p.m., whereas lysis by plasmin in presence of D-α-tocopherol and ethanol was 1,855 c.p.m.

Reported levels of vitamin E in normal human plasma range from 6.5 to  $15 \mu\text{g ml}^{-1}$  ( $1.6$  to  $3.3 \times 10^{-5}$  M)<sup>18</sup>, concentrations greater than those producing 50% inhibition of

**Fig. 2** Effects of varying concentrations of vitamin E on lysis of <sup>125</sup>I-fibrin by plasmin ( $5 \mu\text{g ml}^{-1}$ ). Assay conditions identical to those described in the legend to Fig. 1.



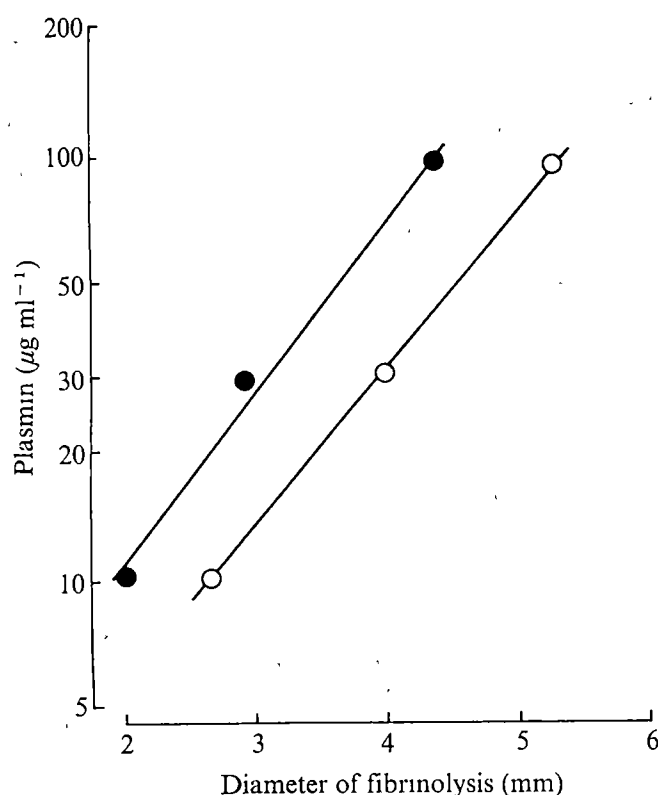


Fig. 3 Effect of vitamin E on lysis of fibrin by plasmin. Plasmin (10, 30 and 100  $\mu\text{g ml}^{-1}$ ), with or without vitamin E ( $1.5 \times 10^{-5}$  M) was introduced into wells (5  $\mu\text{l}$  per well) of heat-treated fibrin plates (Enzo-diffusion plates, Hyland). Diameter of lysis was measured after incubation at 37 °C for 6 h. Semilogarithmic plot plasmin concentration against diameter of lysis, with (●) and without (○) vitamin E. Operational zero point for the assay (no detectable lysis) is 2 mm, the diameter of the wells.

plasmin *in vitro* (Figs 1 and 2). Mean plasma levels of plasminogen-plasmin, determined immunochemically, are approximately 200  $\mu\text{g ml}^{-1}$  (ref. 19). Although comprehensive data on relative proportions of proenzyme and active enzyme in normal plasma are lacking, it is generally accepted that almost all is in the proenzyme form<sup>20</sup>. In the present study, for example, plasminogen-plasmin, freshly prepared from normal plasma<sup>8</sup>, released 360 c.p.m. from <sup>125</sup>I-fibrin, representing less than 2% of the activity (18,911 c.p.m. released) of the same preparation (enzyme concentration, 5  $\mu\text{g ml}^{-1}$ ) after maximal urokinase activation. This estimated plasmin concentration (2% of 200  $\mu\text{g ml}^{-1}$ , or 4  $\mu\text{g ml}^{-1}$ ) is of the same order as the concentrations examined here, and agrees with the observations of others<sup>21</sup>. Relative to other inhibitors, vitamin E produces 50% inhibition at the same concentration (approximately  $10^{-3}$  M) as EACA or tranexamic acid (*trans*-4-aminomethylcyclohexanecarboxylic acid)<sup>8</sup>.

These observations may be pertinent to certain physiological and pathological phenomena, and to vitamin E therapy. Plasmin is normally inhibited by anti-plasmins in plasma. These include such macromolecules as  $\alpha_2$ -macroglobulin,  $\alpha_1$ -antitrypsin and C1 esterase inhibitor<sup>22</sup>. The inhibition of plasmin at physiological concentrations of vitamin E and enzyme raises the possibility that the vitamin may play a role in normal modulation of fibrinolysis. In addition, considering the broad substrate specificity of plasmin for many tissue proteins in addition to fibrin<sup>5</sup>, this effect may be relevant to foetal resorption in vitamin E deficient animals (that is, unopposed proteolytic activity), which led to the initial recognition of this vitamin<sup>23</sup>. The *in vitro* inhibition of plasmin provides an explanation for the reported blunting, on oral vitamin E therapy, of the physiological increase in fibrinolytic activity after venous

occlusion<sup>7</sup>, a response considered to reflect increased conversion of plasminogen to plasmin, mediated by tissue activators released, presumably from vascular endothelium, in response to hypoxia<sup>20</sup>. Further, fibrinolysis has been implicated in the development and spread of tumours<sup>21</sup>. Both EACA and vitamin E inhibit fibrinolysis. Administration of EACA decreases metastatic spread of transplantable tumours in rabbits and rats<sup>24</sup>, and there is a decreased incidence of carcinogen-induced lung tumours in vitamin E deficient mice when compared with normal mice, or deficient mice receiving vitamin E replacement<sup>25</sup>. Finally, in view of the widespread use of the vitamin and its succinate and acetate esters, and the observed effects of oral administration on fibrinolysis *in vivo*<sup>7</sup>, the observations presented here may have implications for the consequences of dietary supplementation with the vitamin.

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## Confusion between specific and nonspecific binding of carcinoembryonic antigen and blood-group antigens by eluted antibody preparations

Cross reactivity between carcinoembryonic antigen (CEA) and blood-group antigens is inferred from the observation that many human sera can bind labelled CEA. This is more common with sera of blood group O or B (that is containing anti-A antibodies), and binding may sometimes be inhibited by absorption of the sera with group A erythrocytes<sup>1,2</sup>.

Following up these observations, Holburn *et al.*<sup>3</sup> showed that hyperimmune human antisera against A, B, Le<sup>a</sup> and Le<sup>b</sup> antigens could bind 30–75% of added labelled CEA. They concluded that the extent of binding suggested that both CEA and blood-group antigens were present on the same molecule. Freedman, commenting on these experiments, pointed out that a hyperimmune antiserum will contain many other "sticky" substances as well as antibodies, and these might account for the results (paper read at meeting of British Society for Immunology, autumn,

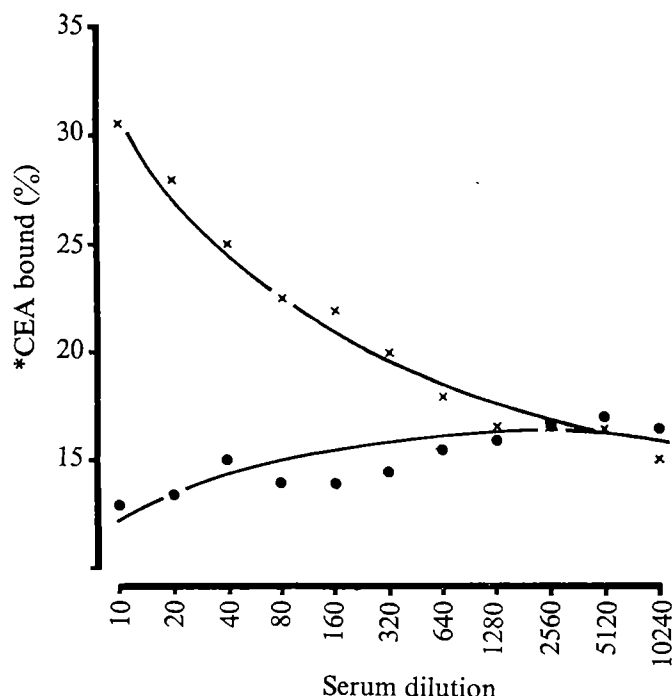


Fig. 1 Binding of untreated anti-A (x) and anti-B (●) to labelled \*CEA, using Farr technique for separation.

1973). To exclude any extraneous binding substances his group in Montreal used "natural" anti-A iso-antibodies, which were adsorbed on to group A erythrocytes and eluted therefrom before use. Such antibody preparations could bind at least 45% of added labelled CEA as judged by the Farr technique<sup>1</sup> and at least 11% as judged by a solid-phase technique<sup>4</sup>. We have now found that rather than removing nonspecific binding capacity from antisera, the process of adsorption to red cells and elution may increase it.

A preparation of CEA, given by Professor A. M. Neville, Chester Beatty Institute, was labelled with <sup>125</sup>I by the chloramine T method<sup>5</sup>. Human anti-A and anti-B sera were obtained from Ortho Diagnostics (lots Pearl 02N0143 and Sally 11M1219, respectively). Samples of 60 ml of each were decomplexed at 56 °C for 30 min and added to 10 ml of washed packed fresh human erythrocytes; anti-A with A cells, anti-B with B cells. After overnight incubation at 4 °C, with gentle mixing, the massively agglutinated cells were washed three times with large volumes of cold 0.9% saline. They were then mixed with an equal volume of 20% bovine serum albumin in saline and gently agitated in a water bath at 56 °C for 10 min to elute the bound antibodies. The supernates were tested by haemagglutination to confirm the presence and specificity of the antibodies.

Each solution was then divided into three equal portions; one to be adsorbed overnight at 4 °C with 10 ml washed packed A erythrocytes, one for similar absorption with group B erythrocytes and one to remain unabsorbed. The cells used for adsorption-elution and for absorption were taken from the same units of blood. Six solutions thus resulted: (1) eluted anti-A antibodies unabsorbed; (2) eluted anti-A antibodies absorbed with group A cells; (3) eluted anti-A antibodies absorbed with group B cells; (4) eluted anti-B antibodies unabsorbed; (5) eluted anti-B antibodies absorbed with group A cells; (6) eluted anti-B antibodies absorbed with group B cells.

The original anti-A and anti-B sera were used, without treatment, as further controls. Haemagglutination tests for antibody in all solutions demonstrated the presence and absence of the expected specificities.

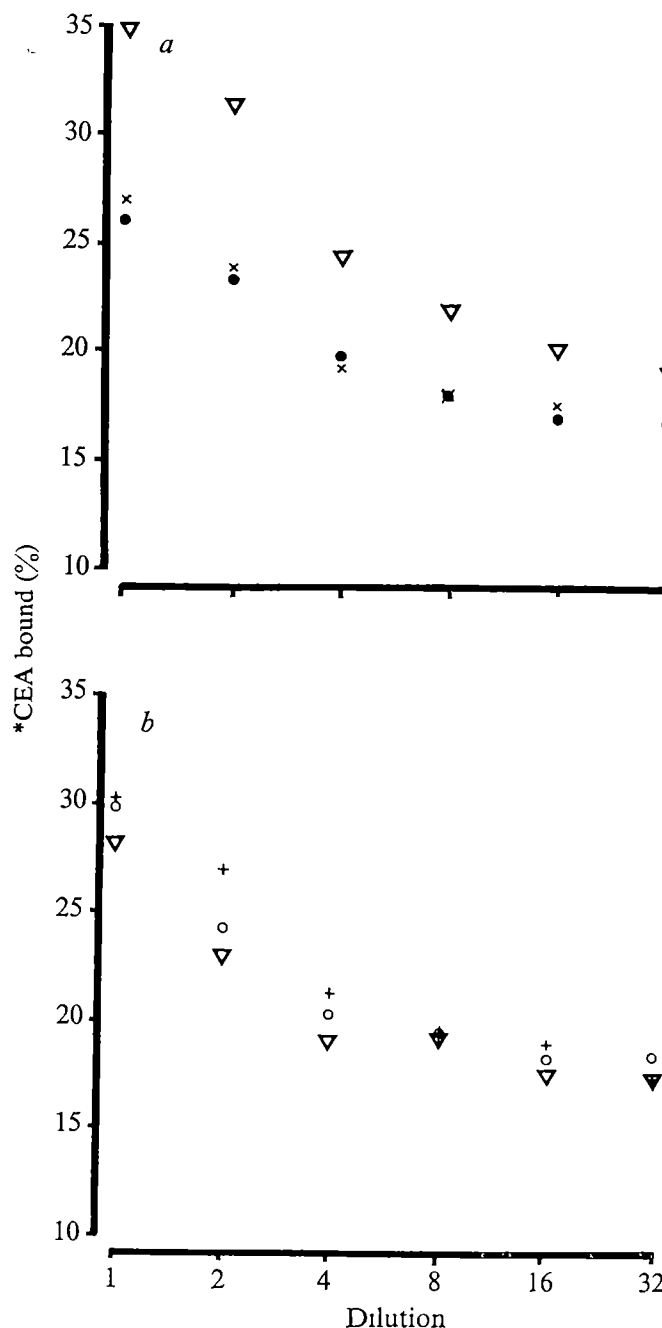
All sera were titrated against labelled CEA, the separation

system being half-saturation with ammonium sulphate in the presence of 0.5 ml of 0.1% bovine gamma globulin. The experiment was repeated on three occasions with the same results.

Figures 1 and 2 show that fresh untreated anti-A can bind at least 30% of the CEA (16% of the binding is nonspecific) while untreated anti-B has no significant activity—if anything, binding is slightly inhibited at a high concentration of serum. After adsorption and elution, all antibody solutions bind CEA to the same extent, irrespective of any subsequent absorption.

It might be argued that the Farr technique (half-saturation with ammonium sulphate) is an inappropriate separation method for this experiment, since a constant 10–20% of CEA itself is precipitated, without any anti-serum, in the presence of a 'carrier' protein (in this case,

Fig. 2 Binding of adsorbed-eluted anti-A and anti-B to labelled CEA, using Farr technique for separation: a: ▽, Anti-A absorbed with A cells; x, anti-A absorbed with B cells; ●, anti-A unabsorbed. b: +, anti-B unabsorbed; ○, anti-B absorbed with A cells; ▽, anti-B absorbed with B cells.



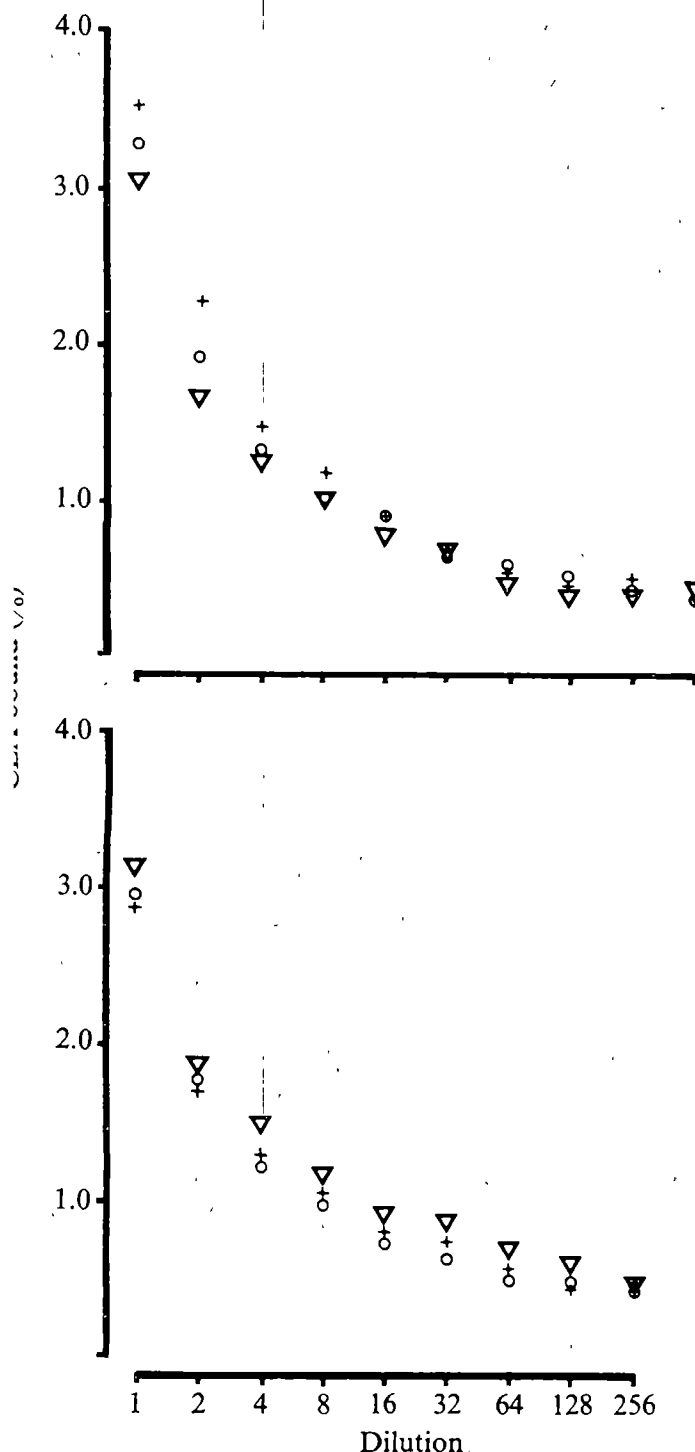


Fig. 3 Binding of adsorbed-eluted anti-A and anti-B to labelled CEA, using insoluble antibody preparations. *a*: +, Anti-A unabsorbed; O, anti-A absorbed with B cells; V, anti-A absorbed with A cells. *b*: +, anti-B absorbed with B cells; O, anti-B absorbed with A cells; V, anti-B unabsorbed

bovine gamma globulin). Separation is therefore not clean and changes in binding capacity of the sera might be hidden. To answer this criticism, the six eluted antibody solutions were precipitated by 18% sodium sulphate and the globulins linked to microcrystalline cellulose (Sigma-Cell type 20) using the cyanogen bromide method<sup>6</sup>. The immunosorbents thus made were titrated against labelled CEA. Figure 3 shows that again there is no essential difference between adsorbed and unadsorbed eluates.

These results show that adsorption and elution of anti-A

from group A red cells makes no difference to its ability to bind CEA, thus confirming the results of Gold *et al.*<sup>1,4</sup>. On the other hand, absorption of this anti-A-containing eluate with group A red cells fails to reduce the binding capacity although its anti-A content becomes undetectable. This indicates that the CEA binding capacity of the eluate is not due to its anti-A activity. This conclusion is further supported by the development of CEA-binding capacity, previously absent, when anti-B is eluted from B cells. Again, this binding is not influenced by further absorption with either A or B cells.

The most likely interpretation is that human erythrocytes contain a 'sticky' non-antibody substance, extractable by heat elution, not removable by absorption with erythrocytes, which can bind to CEA. This may be the explanation for the finding<sup>7</sup> that plasma which had been stored in contact with its cells had a higher apparent CEA level than the same specimen when fresh, and the subsequent finding<sup>8</sup> that a CEA-like substance could be extracted from erythrocyte stromata. It must be remembered that the double antibody technique which was used would register a CEA-binding substance as if it were CEA.

The results reported here also throw doubt on the existence of cross reactivity between CEA and blood-group substances, since they discredit the use of erythrocyte-eluted antibodies for binding studies. Several reports favour such a negative view: (1) a monospecific anti-CEA serum does not agglutinate group A erythrocytes, and blood-group substances A and B do not inhibit CEA-anti CEA binding<sup>1</sup>; (2) *N*-acetyl-D-galactosamine, the immunodominant sugar of A antigen, is conspicuously absent from most CEA preparations<sup>4</sup>; (3) treating CEA with anti-A and anti-i immunosorbents causes no reduction in its CEA activity<sup>9</sup>; and (4) although the blood-group precursors I and i can be found in extracts of colonic cancers, they can be separated from CEA by gel filtration<sup>10</sup>.

An alternative explanation is suggested by the association of CEA with mucus and the mucous coats of cells, both neoplastic and normal (to be published). One of the most striking physical properties of mucus is its cohesiveness. A CEA-binding substance is exuded by colonic cancer cells in tissue culture<sup>2</sup>, and such cells are also known to exude CEA into the medium (as judged by techniques which do not confuse CEA-binding substances with CEA<sup>11</sup>). Erythrocytes possess a mucous coat which they shed into the plasma as they become aged<sup>12</sup>. We would suggest that both CEA and CEA-binding substance reside in mucus and in the mucous coats of cells, though not necessarily on the same molecule.

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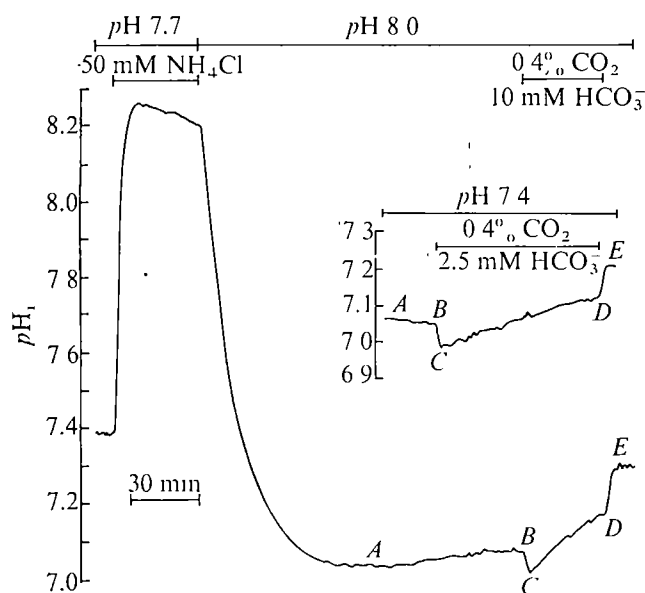
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## Active proton transport stimulated by $\text{CO}_2/\text{HCO}_3^-$ , blocked by cyanide

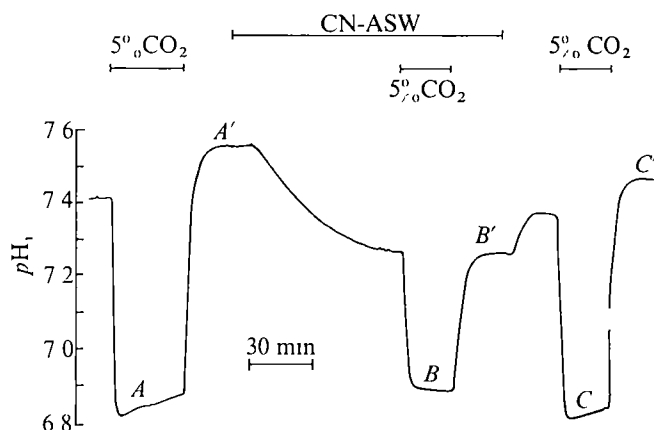
EXPOSURE of a cell to increased  $\text{CO}_2$  levels causes a drop in the intracellular pH ( $\text{pH}_i$ ) (ref. 1). The time development of this acidification has been monitored in snail giant neurones<sup>2</sup> and squid giant axons<sup>3</sup> by means of glass microelectrodes. It was found that  $\text{pH}_i$  does not simply fall to a new steady value during  $\text{CO}_2$  exposure, but, given sufficient time, will slowly recover; and that, on return to a  $\text{CO}_2$ -free solution, a higher  $\text{pH}_i$  value (more alkaline) than the initial one is attained. The slow, secondary alkalisation observed during exposure to  $\text{CO}_2$  cannot be accounted for by any passive mechanism, since the electrochemical gradients for  $\text{H}^+$ ,  $\text{OH}^-$  and  $\text{HCO}_3^-$  all favour further acidification. For example, during the alkalisation, the external pH ( $\text{pH}_o$ ) in squid axons is typically 7.7 and  $\text{pH}_i$  7.0; hence, the Nernst potentials for  $\text{H}^+$  ( $E_H$ ),  $\text{OH}^-$ , and  $\text{HCO}_3^-$  are all  $\sim -40$  mV, compared with a membrane potential ( $V_m$ ) of  $-60$  mV. Thus, a proton extruding pump, or its equivalent, must be postulated. We now report that the rate of alkalisation is at least partially dependent on the  $\text{CO}_2$  and/or  $\text{HCO}_3^-$  content of the bathing medium, that it is



**Fig. 1** Effect of  $\text{CO}_2/\text{HCO}_3^-$  buffer on pumping rate. Exposure to  $\text{NH}_4\text{Cl}$  causes  $\text{pH}_i$  to rise because of the influx and subsequent protonation of  $\text{NH}_3$ .  $\text{NH}_4^+$  is also permeant, however, and its passive influx (the Nernst potential for  $\text{NH}_4^+$  is greater than the membrane potential throughout exposure) has an acidifying effect. Thus, on return to normal ASW,  $\text{pH}_i$  is offset in the acid direction. The cell recovers from this acid load only slowly when HEPES or other non-volatile buffers are used, whereas recovery is greatly accelerated during exposure to a  $\text{CO}_2/\text{HCO}_3^-$  buffer at the same pH. The prompt acidification occurring at the onset of the  $\text{CO}_2$  exposure (segment BC) is due to the influx, hydration, and subsequent dissociation of  $\text{CO}_2$ . Removal of  $\text{CO}_2$  (segment DE) causes a reversal of these reactions, and therefore a rapid alkalisation<sup>3</sup>. The membrane potential in the post- $\text{NH}_4\text{Cl}$  period was  $-59$ – $-60$  mV; the Nernst potential for  $\text{H}^+$  varied from  $-56$ – $-48$ . In the experiment in the inset, the  $\text{pH}_o$  was 7.4 following a similar pretreatment with  $\text{NH}_4\text{Cl}$ -ASW. In HEPES-ASW the  $\text{pH}_i$  slowly falls (segment AB, rate =  $-0.03$   $\text{pH h}^{-1}$ ) while in  $\text{CO}_2/\text{HCO}_3^-$ -ASW, the  $\text{pH}_i$  rises (segment CD, rate =  $0.12$   $\text{pH h}^{-1}$ ). During the alkalisation  $V_m$  was  $-62$  mV and  $E_H$  was  $-24$ – $-17$  mV. In these experiments, axons were mounted horizontally in a chamber (temperature =  $22^\circ\text{C}$ , volume  $\approx 0.15$  ml) superfused (1 ml  $\text{min}^{-1}$ ) with ASW.  $\text{CO}_2$ -containing solutions were delivered from a glass syringe (after equilibration) through  $\text{CO}_2$ -impermeable tubing. The glass  $\text{pH}_i$  electrodes were of Hinkes's design<sup>4</sup>.

reversibly blocked by metabolic inhibitors and that an extracellular acidification accompanies the intracellular alkalisation.

These experiments were performed on giant axons of the squid *Loligo pealei*, cannulated at both ends to allow for the introduction of pH and reference electrodes. In the experiment shown in Fig. 1, an axon ( $\text{pH}_i$  7.39) was pretreated with artificial seawater (ASW) containing 50 mM  $\text{NH}_4\text{Cl}$  for  $\sim 30$  min. When, after prolonged exposure to  $\text{NH}_4\text{Cl}$ , an axon is returned to ASW, the  $\text{pH}_i$  reaches a value considerably more acid than the resting  $\text{pH}_i$  (ref. 3). In the case of Fig. 1, the  $\text{pH}_i$  fell to 7.03.



**Fig. 2** Secondary alkalisation blocked by cyanide. An axon was first exposed to 5%  $\text{CO}_2/50$  mM  $\text{HCO}_3^-$ -ASW. After the initial acidification, the axon underwent secondary alkalisation (A), on return to normal ASW, the  $\text{pH}_i$  overshoot the initial  $\text{pH}_i$  (A'). Subsequent exposure to nominally 2 mM CN caused the  $\text{pH}_i$  to fall slowly<sup>3</sup>, and to stabilise eventually over the course of 75 min. Exposure to nominally 5%  $\text{CO}_2/50$  mM  $\text{HCO}_3^-$ , CN-ASW caused the abrupt decline in the  $\text{pH}_i$ , characteristic of  $\text{CO}_2$  exposure, but led to no secondary alkalisation (B). Similarly, there was no overshoot of the  $\text{pH}_i$  when the axon was returned to CN-ASW (B'). After recovery in normal ASW, the axon once again was capable of secondary alkalisation in the presence of  $\text{CO}_2$  (C), and exhibited the characteristic overshoot on removal of the  $\text{CO}_2$  (C'). The temperature was  $22^\circ\text{C}$  throughout.

If a proton pump were designed to maintain a relatively alkaline intracellular milieu in the face of acid challenges, then one would expect the acid load following exposure to  $\text{NH}_4\text{Cl}$  to be an effective stimulus for increased pumping. Indeed, during the time segment AB, the  $\text{pH}_i$  rose, though very slowly ( $0.04$   $\text{pH h}^{-1}$ ), when  $\text{NH}_4\text{Cl}$ -free ASW was buffered at pH 8.00 with 0.5 mM HEPES. Replacement of the HEPES buffer with 0.4%  $\text{CO}_2/10$  mM  $\text{HCO}_3^-$ , keeping the seawater pH constant, however, caused the  $\text{pH}_i$  (after a small acidification due to the influx of  $\text{CO}_2$ ) to rise (segment CD) at a much greater rate ( $0.35$   $\text{pH h}^{-1}$ ). The stimulation caused by  $\text{CO}_2/\text{HCO}_3^-$  at pH<sub>o</sub> 7.4 is shown in the inset of Fig. 1. Since in both cases  $E_H > V_m$ , an active process is required to explain the observed alkalisation. In a series of 11 such experiments, the rate of intracellular alkalisation after the treatment with  $\text{NH}_4\text{Cl}$  was, at a given  $\text{pH}_o$ , always far greater in  $\text{CO}_2/\text{HCO}_3^-$ -ASW than in HEPES-ASW. The rate also increased with increasing  $\text{pH}_o$ .

The alkalisation occurring in the presence of  $\text{CO}_2/\text{HCO}_3^-$  could readily be blocked by the metabolic inhibitors cyanide (CN) and 2,4-dinitrophenol (DNP). Figure 2 illustrates an experiment in which an axon, capable of secondary alkalisation (point A) in the presence of  $\text{CO}_2$ , was exposed to CN. After  $\sim 75$  min, when the  $\text{pH}_i$  had reached a steady level, the axon was exposed to a  $\text{CO}_2/\text{HCO}_3^-$  solution in the continued presence of CN. Although typical acidification occurred, no secondary alkalisation was observed (B). Return to cyanide-free ASW and subsequent exposure to  $\text{CO}_2$  showed that the axon was once again capable of secondary alkalisation (C). Similar results were obtained with DNP.

Evidence presented so far does not rule out intracellular  $H^+$  sequestration as the cause of secondary alkalisation in the presence of  $CO_2$ . We determined that intracellular secondary alkalisation is accompanied by extracellular acidification, as follows: after pretreatment with 50 mM  $NH_4Cl$ -ASW, we placed axons in sealed capillaries containing ASW and phenol red, and followed the absorbance at 559 nm. When the ASW contained 10 mM  $HCO_3^-$  ( $pH_o$  8.0), we always observed a decrease in absorbance, corresponding to extracellular acidification; replacing the  $HCO_3^-$  with 10 mM TES ( $pH_o$  8.0;  $pK_a'$  approximately the same as for  $CO_2$ ) caused, if anything, an increase in absorbance. Again, treatment with CN prevented the characteristic absorbance decrease in the presence of  $HCO_3^-$ . Although the spectrophotometric measurements are difficult to quantify, they provide strong evidence that, as acid disappears from the cell interior, acid appears on the outside.

We acknowledge support by the NIH, and a Grass fellowship.

*Note added in proof:* Thomas<sup>5</sup> has observed that the presumed  $H^+$  pump of snail neurones, too, is more active in the presence of  $CO_2$ .

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## The presence of Na-Na and Na-K exchange in sodium extrusion by three species of fish

ALTHOUGH it has been generally assumed that marine teleost fish balance the net influx of NaCl (from diffusional gain and oral ingestion of seawater) by active extrusion of both ions<sup>1-3</sup>, the proposition of active extrusion of Na has been questioned. It has been shown<sup>4-11</sup> that marine teleosts generally maintain a transepithelial potential (t.e.p.) in the range +10-+25 mV (blood relative to seawater), which is nearly equal to that which could nullify the Na concentration gradient between the teleost and seawater. Thus, it has been suggested that Na is maintained in electrochemical equilibrium across the teleost gill and only Cl is

actively extruded<sup>4,6-8,12</sup>. The Na efflux changes accompanying rapid changes in external Na or K concentrations have been used to support a model of both Na-Na and Na-K exchanges<sup>13-16</sup>, and have been shown to result at least partially from changes in the t.e.p. (refs 7-9, 12) rather than uncoupling of ionic exchange systems. Histochemical localisation of the Na-K-activated ATPase in teleost gill tissue indicates that it is predominantly basal rather than apical<sup>17,18</sup>. This suggests that the enzyme may be involved in gill cell volume regulation rather than transepithelial extrusion of Na.

Even if marine teleosts maintain blood Na in electrochemical equilibrium with seawater they face a net Na gain because of oral ingestion of the medium<sup>1,19</sup>. Also, the K-stimulated Na efflux from at least two species of marine teleosts is greater than can be accounted for by changes in the t.e.p. (refs 9, 11). Evans *et al.*<sup>16</sup> have shown that removal of K from the seawater reduces the Na efflux from *Dormitator maculatus* without affecting the t.e.p. The rapid reduction in Na efflux after transfer of a marine teleost to freshwater (or tap water) is secondary to changes in the t.e.p. (refs 7-9, 12), but Kirschner *et al.*<sup>12</sup> have shown that t.e.p. changes can only account for 60% of the fall in Na efflux from the trout, *Salmo gairdneri*, transferred from seawater to Na-free, K-free artificial seawater.

The validity of the model of Na-K and Na-Na exchange across the marine teleost gill is thus questionable, and to try to describe the components of Na efflux from marine teleost fish more clearly, we investigated the kinetics of Na efflux and the changes in t.e.p. in three species of teleosts, *Achirus lineatus* (lined sole), *Hippocampus erectus* (lined seahorse) and *Opsanus beta* (gulf toadfish) in seawater and other electrolyte solutions. All specimens were collected by us on commercial shrimp trawlers in Biscayne Bay, Florida. They were maintained in seawater (500 mM  $Na^+$ ) in 30-gallon glass aquaria at  $22 \pm 1^\circ C$  for at least 1 week before experimentation and were fed either TetraMin (Tetra Werke) or live brine shrimp until 24 h before an experiment. The techniques for determination of rapid changes in unidirectional Na efflux and t.e.p. have been described<sup>9,16</sup>. The only modification was that the t.e.p. was determined on unanaesthetised and submerged sole and toadfish, the seahorse was anaesthetised lightly ( $4 \times 10^{-3}$  g per 1 MS-222) and held with the head and opercula submerged and the site of bridge implantation above the water level. Fluxes and t.e.p. were measured in separate experiments and the fish were either transferred from seawater to tap water or from seawater to K-free, Na-free seawater (-K, -Na SW) and

Table 1 Effect of ion-free solutions on the unidirectional Na efflux and the t.e.p. across three marine teleosts

Species	Na efflux			
	SW	TW	-K, -Na SW	25 K, -Na SW
<i>A. lineatus</i>	100	$46 \pm 3$ (7)*	$18 \pm 4$ (6)*	$29 \pm 4$ (6)†
<i>H. erectus</i>	100	$36 \pm 1.4$ (5)*	$19 \pm 2$ (13)*	$27 \pm 3$ (13)†
<i>O. beta</i>	100	Unstable (10)‡	$11 \pm 3$ (7)	$28 \pm 2$ (7)†
t.e.p.				
<i>A. lineatus</i>	$-6.8 \pm 0.6$ (20)	$+5.5 \pm 1.4$ (6)*	$-15 \pm 0.6$ (5)*	$-15 \pm 0.9$ (5)
<i>H. erectus</i>	$-3.5 \pm 0.4$ (14)	$-1.9 \pm 1.9$ (9)*	$-9.2 \pm 0.4$ (14)*	$-9.3 \pm 0.7$ (14)
<i>O. beta</i>	$-8.1 \pm 0.9$ (12)	Unstable (6)‡	$-15 \pm 1.5$ (6)	$-15 \pm 1.5$ (6)

All data are expressed as mean  $\pm$  s.e., number of experiments in parentheses. All flux values are expressed as a % of the control efflux when the animal is in seawater. The t.e.p. is expressed as blood relative to medium (mV).

\* Significantly different ( $P \leq 0.005$  in paired experiments) from the seawater control.

† Significantly different ( $P \leq 0.005$  in paired experiments) from the -K, -Na SW data. -K, -Na SW: 2.5 mM  $Na^+$ , 571 mM  $Cl^-$ , 53 mM  $Mg^{2+}$ , 10 mM  $Ca^{2+}$ , 28 mM  $SO_4^{2-}$ , 2.5 mM  $HCO_3^-$ , 501 mM choline<sup>2+</sup>. 25 K, -Na SW; same as above except that 25 mM  $K^+$  is added as the sulphate salt.

‡ Effluxes and t.e.p. of *O. beta* in tap water are unstable but show the same trends as displayed in the other two species. In tap water the sodium efflux falls to  $32 \pm 2.7\%$  of the seawater control but within 3-5 min increases to  $81 \pm 5.5\%$  of the control. In tap water the t.e.p. always depolarises (range -1 to +16 mV) immediately, but within 10 min falls back towards the t.e.p. in seawater and stabilises at a t.e.p. usually somewhat depolarised from the seawater control (range -6 to +6 mV).

then into 25 mM per l K-enriched, Na-free seawater (25 K, -Na SW).

The results are shown in Table 1. All three species maintain their blood distinctly negative to seawater and this is the first description of a marine teleost with t.e.p. blood negative to seawater. Although we did not determine blood Na concentrations in our animals, all marine teleosts have a blood Na concentration well below that of seawater<sup>20</sup> so it seems that, unlike other teleosts, *A. lineatus*, *H. erectus* and *O. beta* face a substantial electrochemical gradient for Na across their epithelium. They all display a significant reduction in Na efflux and change in t.e.p. after transfer to tap water. In all cases, however, the t.e.p. becomes less negative rather than more negative as has been described for other teleosts<sup>7-9,12</sup>. Obviously, changes in the t.e.p. cannot account for the observed reduction in unidirectional Na efflux from these species in tap water. So it seems that the decline in unidirectional Na efflux is secondary to an uncoupling of Na-K and/or Na-Na exchange rather than a change in passive efflux. Tap water, however, is not only Na- and K-free but also free of other ions (for example, Ca, Mg) that may be important in maintenance of the normal marine teleost gill epithelium, and it has been shown that external Ca has profound effects on the fluxes of Na and Cl as well as the t.e.p. in both the freshwater goldfish, *Carassius auratus* and the marine mullet, *Mugil capito*<sup>21,22</sup>. Thus, it is more relevant to measure the flux and t.e.p. changes after transfer from SW to -K, -Na SW rather than to tap water. In all three species, transfer to -K, -Na SW results in a 6-8-mV increase in the internal negativity and an 80-90% reduction in unidirectional Na efflux. Although the change in the t.e.p. could account for a 10% reduction in Na efflux (see refs 9 and 12 for relevant formulae), it cannot account for the reduction seen experimentally.

The discrepancy between the data in tap water and -K, -Na SW should be noted (especially for *O. beta*). With the exception of that by Kirschner *et al.*<sup>14</sup>, other studies of flux and t.e.p. changes in ion-free media have involved the use of tap or freshwater. It can be seen from our work that transfer of a marine teleost to tap water may alter the permeability characteristics of the gill epithelium sufficiently to produce spurious results.

Thus, our data clearly show that the majority of the unidirectional Na efflux from three marine teleosts is coupled to the external K and/or Na concentration exclusive of t.e.p. changes. Data in Table 1 show clearly that, after transfer from -K, -Na SW to 25K, -Na SW, although the t.e.p. does not change, the Na efflux increases in all three species. Thus, Na-K exchange does occur in Na extrusion in these teleosts but (since even 25 mM K l<sup>-1</sup> results in an Na efflux of only 30% of the Na efflux in seawater) is a relatively minor component compared with diffusional efflux and Na-Na exchange. In summary, *A. lineatus*, *H. erectus*, and *O. beta* maintain their blood Na out of electrochemical equilibrium with seawater Na and extrude the excess Na by way of an Na-K exchange mechanism. In addition, Na-Na exchange diffusion is important in the unidirectional Na efflux from these species.

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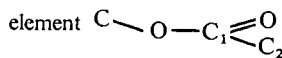
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## Roles of carbonyl oxygens at the bilayer interface in phospholipid-sterol interaction

FROM many recent studies on the phospholipid-sterol interaction, it has become increasingly clear that the 3  $\alpha$ - and 3  $\beta$ -hydroxy isomers of a range of sterols have quite different effects on the molecular mobility and functional property of lipid bilayer membranes<sup>1,2</sup>. For example, the addition of cholesterol or  $\Delta^5$ -cholesten-3  $\beta$ -ol to liposomes decreases their permeability, whereas epicholesterol, a 3  $\alpha$ -isomer of cholesterol, exerts no apparent effect. It has been proposed that the 3  $\beta$ -hydroxyl group engages in hydrogen bonding with the carbonyl oxygen of the fatty acyl groups in phospholipids in the bilayer<sup>1</sup>. Evidence supporting such hydrogen bond formation is strong<sup>4</sup>. The problem is, then, why does the 3  $\beta$ -OH group of cholesterol, but not the 3  $\alpha$ -OH group of epicholesterol form such bonds?

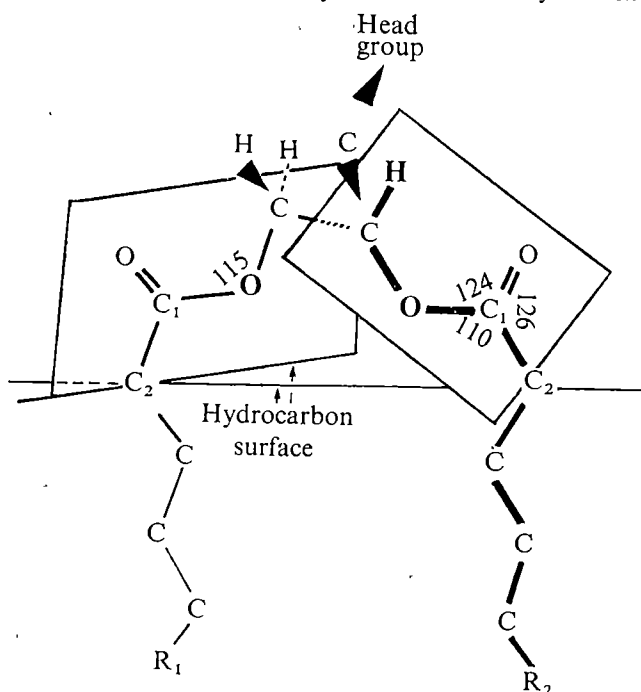
Here I first discuss the molecular configuration of the bilayer interface and its characteristics, and then propose molecular models to describe hetero-intermolecular hydrogen bonds between the carbonyl groups of phospholipids, hydroxyl groups of sterols, and water molecules in the bilayer interface, which enable rationalisation at the difference in activity between the 3  $\alpha$ - and 3  $\beta$ -hydroxy isomers of sterols.

Fig. 1 Schematic diagram showing the planarity of the structural

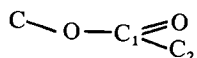


of a phospholipid molecule and the

hydrocarbon surface of the bilayer. Values of various bond angles are taken from the crystal structure of acetylcholine<sup>5</sup>.



The small region taken up by the structural element



of phospholipids in the bilayer is termed the bilayer interface. Here, C1 and C2 are, respectively, the first and second carbon atoms of the fatty acyl chain. Several aspects of the geometry of the elements are pertinent. First, a plane passing through all the C2 carbons of the acyl chains can be considered as the hydrocarbon or non-polar surface of the bilayer (Fig. 1). Second, the carbonyl double bond and the partial double bond character of the ester C-O bond cause all carbon and oxygen atoms in the structural element to be coplanar<sup>5,6</sup>, similar to the atomic arrangements found in the amide plane of polypeptide chains and sphingolipids. Moreover, the C-H bond of the secondary ester is synplanar to C1=O as shown in Fig. 1<sup>6,7</sup>. Because of this planarity of the structural element, the segmental motions of carbon atoms in the bilayer interface are expected to be rather restricted. Indeed, <sup>13</sup>C-NMR (nuclear magnetic resonance) experiments demonstrate that the spin-lattice relaxation times of carbon nuclei within the phosphatidylcholine molecule in a bilayer increase from the glycerol backbone to both the methyl ends of the two fatty acyl chains and the -N<sup>+</sup>(Me)<sub>3</sub> end of the polar head group, indicating the smallest segmental mobility of the carbon atoms in the structural element<sup>8,9</sup>. Finally, on the basis of the planarity of the structural element together with the vertical orientation of the two parallel fatty acyl chains away from the hydrocarbon surface in the liquid crystalline state<sup>10</sup>, it is reasonable to assume that the C1=O bond adopts a tilted orientation with respect to the hydrocarbon surface as shown in Fig. 1. In spite of the uncertainty in the precise orientation of the carbonyl groups, it is important to point out that the carbonyl group is held rigidly in proximity to the hydrocarbon phase of the bilayer which has a low dielectric constant.

In discussing the carbonyl oxygen in the bilayer interface serving as a hydrogen bond acceptor, two features of hydrogen bonds must be considered. In the first place, of 196 hydrogen bond angles studied, the configuration of O-H...O bond angles which appeared with the greatest frequency was found to be approximately 15° from the linear configuration<sup>11,12</sup>. In other words, a stable hydrogen bond has such a configuration that the donor, hydrogen, and acceptor atoms are nearly colinear. Second, the hydrogen bond is electrostatic in origin, with greater strength in a medium of low dielectric constant. The intrapeptide hydrogen bonds, for example, do exist in the interior of proteins where the effective dielectric constant is relatively low.

A plausible model for a phospholipid-cholesterol complex at the interface of a bilayer is presented in Fig. 2a, showing how the equatorial hydroxyl group on C3 can satisfy the necessary conditions for hydrogen bond formation. The rigid, planar fused ring system of the molecule

is inserted vertically into the hydrocarbon core with C3 at the hydrocarbon surface, bringing the more polar hydroxyl group into the interface, where the dielectric constant is still sufficiently low to enhance the formation of a hydrogen bond.

It is not likely that water can displace and thus compete with cholesterol for the formation of stable hydrogen bonds to carbonyl oxygens at the same binding site as shown in Fig. 2a, because the necessary orientation would bring water molecules near the hydrocarbon phase, an energetically improbable circumstance. Water molecules may, however, form bonds by orientating themselves in solution towards the polar surface of the bilayer. This possibility would be favoured by the presence of cholesterol, which increases the separation of head groups on the phospholipid molecules<sup>13</sup>, and therefore also increases the configurational freedom of water molecules in the upper portion of the bilayer interface. Thus, my model predicts that no more than one molecule of water will form hydrogen bonds with each carbonyl oxygen atom of the bilayer (two per molecule of phospholipid) in any event, and bonding may be even more difficult in the absence of cholesterol.

The model explains why sterols with the 3α configuration lack activity in membrane functions. Figure 2b illustrates the orientation of epicholesterol, the 3α isomer of cholesterol, when it is positioned so as to bring the hydroxyl group into the interface; Fig. 2b shows that the O-H...O bond angle is approximately 55°, too great for effective hydrogen bonding<sup>12</sup>. Lowering the sterol so as to bring carbon 3 to the same level as that shown for cholesterol has the effect of not only increasing the distance between the OH group and the carbonyl oxygen which is unfavourable for hydrogen bond formation, but also partially embedding the hydroxyl oxygen atom into the hydrocarbon region of the bilayer which is energetically unfavourable.

In summary, I believe the key difference between the effect of cholesterol and epicholesterol on the bilayer structure and function lies in their hydrogen-bonding abilities toward the carbonyl group of the ester linkage of the acyl side chains of phospholipids. This difference is a natural consequence of the constrained orientation of these carbonyl oxygen atoms. It is well known that the polar head group of cholesterol molecule may dictate the orientation of the whole molecule in the monolayer<sup>14</sup>. I would like to point out that the hydrogen-bonded structural effect of cholesterol may also lead to long range effects on the packing density of the hydrocarbon chains of neighbouring phospholipids. Such changes in hydrocarbon packing would be expected to affect the permeability properties of the bilayer membrane<sup>1</sup>. Furthermore, a simple substitution of the hydrogen bond donor from cholesterol shown in Fig. 2a to proteins affords a reasonable extension of the molecular model to explain some of the lipid-protein interaction. In fact, <sup>13</sup>C chemical shifts of carbonyl groups of different classes of lipoproteins have been reported<sup>15</sup>. These observed chemical shifts are consistent with a molecular model in which carbonyl oxygens of lipids are engaged in hydrogen bonding to water molecules and other membrane components<sup>3</sup>.

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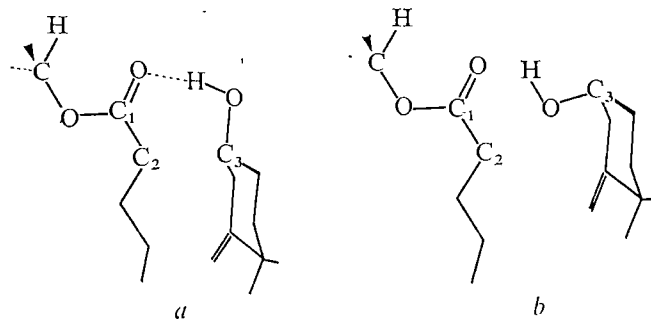
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Fig. 2 Plausible spatial orientation of cholesterol (a) and epicholesterol (b) in the bilayer interface. Hydrogen-bond formation between the phospholipid carbonyl group and cholesterol hydroxyl group is given in (a) as a dashed line.





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## Effect of prostaglandin synthesis on renal function and renin in the dog

THERE is considerable evidence for an interrelationship between the prostaglandin and renin-angiotensin systems in the kidney. Infusion of angiotensin II into the renal arteries of dogs and rabbits releases a vasodilator prostaglandin-like material into the renal vein<sup>1-4</sup>. It also seems that prostaglandins (PGs) induce renin release in the kidney. Riley and his coworkers<sup>5,6</sup> infused PGE<sub>1</sub> in to the dog renal artery and observed increases in plasma renin activity (PRA) of renal vein blood and in renin secretion. Elevated PRA was observed in blood from the inferior vena cava, just above the entrance of the renal vein, after aortic infusions of PGE<sub>1</sub> (ref. 7) and infusion of PGE<sub>1</sub> and PGA<sub>1</sub> in to the stenosed renal arteries of hypertensive dogs, caused an elevation of renal vein renin<sup>8</sup>. PGE<sub>2</sub> was shown to stimulate renin release in a rabbit renal cortex cell suspension<sup>9</sup>. Indomethacin decreased the PRA of normal and hypertensive rabbits<sup>10,11</sup>, and prevented the elevation of PRA in rabbits with glycerol-induced acute renal failure<sup>12</sup>. In contrast, however, Vander<sup>13</sup> infused both PGE<sub>1</sub> and PGE<sub>2</sub> into the dog renal artery and failed to observe an effect on renin output.

More recently, Larsson *et al.*<sup>11</sup> infused sodium arachidonate, the precursor of PGE<sub>2</sub> and PGF<sub>2α</sub>, into the descending aorta of the rabbit and observed elevated PRA in blood from the

inferior vena cava. Their control PRA levels were, however, abnormally high (which they attributed to the anaesthetic), and neither renin secretion, urinary parameters, nor renal vein PG levels were measured during the sodium arachidonate infusion. Anggard and Larsson<sup>14</sup> stimulated renin release by the addition of arachidonic acid to an *in vitro* rabbit kidney preparation. In our study we examined the effects of sodium arachidonate-induced changes in renal function on renin output.

The increased renal blood flow (RBF) caused by the infusion of sodium arachidonate is shown in Table 1. There were no significant changes in the mean glomerular filtration rates (GFR) of the three infusion periods or the GFR of the three urine collections in the sodium arachidonate period. Since the GFR was not altered, the calculated filtration fraction decreased, suggesting predominantly efferent arteriolar dilatation. The increase in RBF occurred in the absence of any systemic effects and was characterised by a delay in onset of 31.5±18.2 s (s.e.m., *N* = 11). Coincidental with the increase in RBF there was a marked diuresis and natriuresis. In the first control period the urinary flow rate and sodium excretion rate for the right kidney were lower than those of the left. This is probably the result of surgical trauma, because the right kidney was unavoidably manipulated and the left kidney was untouched. The contralateral kidney demonstrated no significant alterations during the infusion of sodium arachidonate. The haemodynamic, diuretic and natriuretic responses are similar to those reported by others who infused PGE<sub>2</sub> and sodium arachidonate into dog renal arteries<sup>15,16</sup>. Accompanying the urinary and RBF changes were significant increases in renal vein PRA, renin secretion and renal vein PGF material. The PGF levels were used as indicators of increased activity of the arachidonate-prostaglandin system, since sodium arachidonate has been shown to stimulate synthesis and release of both PGE and PGF by the kidney<sup>15,17</sup>.

All responses to sodium arachidonate infusion were markedly attenuated by pretreatment with indomethacin. The inhibition by indomethacin of the sodium arachidonate-induced elevation

Table 1 Effects of sodium arachidonate on renal function in dogs

Infusion periods	$V$ ( $\mu\text{l min}^{-1} \text{g}^{-1}$ )				$U\dot{V}_{\text{Na}^+}$ ( $\mu\text{Eq min}^{-1} \text{g}^{-1}$ )				FF		RBF ( $\text{ml min}^{-1} \text{g}^{-1}$ )		Renal vein PRA ( $\text{ng ml}^{-1} \text{h}^{-1}$ )		Renin secretion ( $\text{ng min}^{-1} \text{h}^{-1}$ )		Renal vein PGF ( $\text{pg ml}^{-1}$ )	
	L (16)	R (16)	L (7) PI	R (7) PI	L (16)	R (16)	L (7) PI	R (7) PI	R (16)	R (7) PI	R (16)	R (7) PI	R (10)	R (7) PI	R (6)	R (5) PI	R (7)	R (5) PI
Control	41 ±6	33 ±6	16 ±5	15 ±4	3.0 ±0.4	1.9 ±0.4	1.2 ±0.5	1.1 ±0.4	0.27 ±0.03	0.32 ±0.03	4.1 ±0.4	4.9 ±0.5	4.2 ±0.6	2.1 ±0.4	94.3 ±32.8	73.8 ±29.9	181 ±60	200 ±22
Sodium arachidonate	40 ±6	51* ±7	12 ±3	19 ±6	3.2 ±0.5	3.3* ±0.5	1.3 ±0.3	1.7 ±0.6	0.19* ±0.02	0.31 ±0.03	5.8* ±0.5	5.1 ±0.5	7.6† ±1.3	2.6 ±0.6	473.8† ±107.5	100.9 ±48.7	443† ±123	268 ±87
Control	34 ±5	32 ±4	13 ±3	11 ±3	3.4 ±0.5	2.5 ±0.4	1.4 ±0.3	1.2 ±0.4	0.27 ±0.03	0.28 ±0.01	4.0 ±0.4	4.8 ±0.3	3.9 ±0.6	3.3 ±0.8	101.1 ±46.1	132.0 ±64.3	229 ±109	178 ±58

The effects on renal function of sodium arachidonate in salt-loaded dogs and salt-loaded dogs pretreated with indomethacin (PI). Values are given as the mean ± s.e.m. Number in parentheses are the number of animals. R, Right (infused) kidney; L, left (control) kidney;  $\dot{V}$ , urinary flow rate;  $U\dot{V}_{\text{Na}^+}$ , urinary sodium excretion rate; FF, filtration fraction; \**P* < 0.001; †*P* < 0.01; ‡*P* < 0.05. Twenty-three female mongrel dogs were fasted 18 h before surgery and allowed free access to water. Anaesthesia was induced by intravenous pentobarbital. Indwelling polyethylene catheters were inserted into the right and left femoral arteries for monitoring systemic blood pressure and collecting blood samples, respectively. A catheter was also placed in the left femoral vein and advanced into the right renal vein. A fourth catheter was placed in the right femoral vein for the infusion of inulin. A suprapubic midline abdominal incision was made and both ureters cannulated. A right retroperitoneal flank incision was made, and a pneumatic cuff and electromagnetic flowprobe were placed around the renal artery. The pneumatic cuff was used to establish and verify the electronic zero flow. RBF was monitored by a sine wave, non-occluding electromagnetic flowmeter (Biotronex Laboratories). A 25 gauge infusion needle was inserted into the right renal artery, normal saline was infused at 0.5 ml min<sup>-1</sup>. The animals received an intravenous saline load of 10% body weight, after which the intravenous saline infusion was matched to the urine output of 0.5-2.0 ml min<sup>-1</sup>. Seven animals received an intravenous injection of indomethacin at a dose of 10 mg kg<sup>-1</sup> buffered to pH 7.4. One hour was allowed before the commencement of the experiment. The study was comprised of three 30-min periods; three 10-min urine collections were obtained during each period. Midway through each period arterial and renal vein samples were collected for inulin, sodium, renin and PGF determinations. After the first control period, sodium arachidonate<sup>28</sup> (Nu Chek Prep) was infused into the renal artery, delivering 25  $\mu\text{g}$  per kg body weight per min. Thirty minutes was allowed for renal function to return to control levels and then the final control period was begun. The animals were killed and the kidneys removed and weighed. Renin and PGF levels were determined by radioimmunoassay. The level of significance was determined by the paired Student's *t* test.

in renin secretion indicates that sodium arachidonate is not active *per se* but must be converted to another compound, such as one of the intermediary endoperoxides, thromboxane  $A_2$  and/or PGs.

There are several possible explanations for the observed increase in renin release. Both renal nerve stimulation and the infusion of catecholamines into the renal circulation enhance renin release<sup>18,19</sup>, and PGF has been shown to potentiate adrenergic activity<sup>20,21</sup>. Frame *et al.*<sup>22,23</sup>, however, observed that PGE<sub>2</sub> and sodium arachidonate attenuate renal sympathetic transmitter release and vascular responses to noradrenaline in the rabbit kidney. Therefore, it is doubtful that the sympatho-adrenal system is involved in the sodium arachidonate-induced renin release. Riley *et al.*<sup>5</sup>, concluded that the PGE<sub>1</sub>-induced elevation of renin output was due to vasodilatation and physical distortion of the juxtaglomerular apparatus. Tagawa and Vander<sup>24</sup>, however, found that vasodilatation induced by acetylcholine had no effect on renin release. Furthermore, Witty *et al.*<sup>25</sup> observed no significant alterations in renin secretion when papaverine, a potent vasodilator, was infused into the renal artery of the denervated non-filtering kidney, the denervated filtering kidney and the innervated non-filtering kidney of the dog. This suggests that renal vasodilatation or juxtaglomerular apparatus distortion do not necessarily result in increases in renin secretion. In addition, several investigators have shown that decreases in renal perfusion pressure which result in decreased afferent arteriolar stretch stimulate renin secretion<sup>26,27</sup>. Werning *et al.*<sup>7</sup> and Varkarakis *et al.*<sup>8</sup> attributed the stimulation of the renin-angiotensin system by PGE<sub>1</sub> to alterations in the sodium concentration in the tubular urine acting through the macula densa. In contrast, Tagawa and Vander<sup>24</sup> demonstrated that acute water and electrolyte losses induced by renal vasodilatation did not result in elevations in renin secretion. These observations indicate that distortion of the juxtaglomerular region, changes in sodium flux at the macula densa or adrenergic stimulation are not primarily responsible for the increase in renin release seen with the infusion of sodium arachidonate. PGE<sub>2</sub> and arachidonate stimulation of renin release in the *in vitro* rabbit kidney preparation also argue against these theories. We, therefore, favour the concept that the arachidonate-PG system stimulates renin secretion by a direct action on the juxtaglomerular apparatus in the intact animal.

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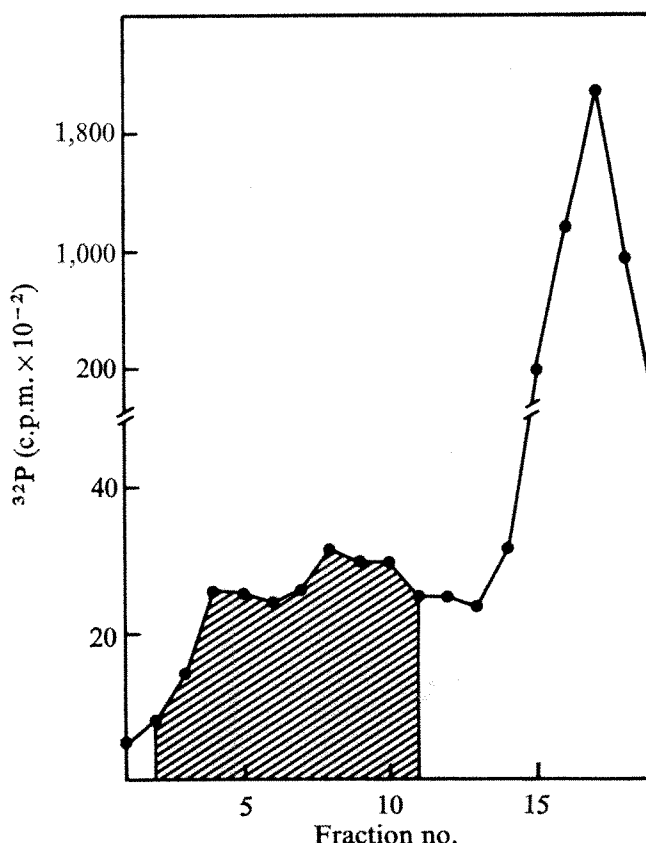
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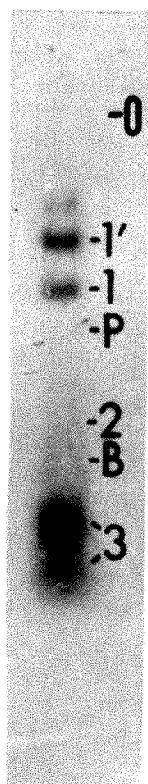
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## Interaction of tryptophan transfer RNA with Rous sarcoma virus 35S RNA

THE primer for the initiation of DNA synthesis by the RNA-directed DNA polymerase (reverse transcriptase) on Rous sarcoma virus (RSV) 70S RNA has been identified as tryptophan tRNA<sup>1,2</sup>. The RNA primer hybridises efficiently to 35S RNA subunits produced by denaturation of 70S RNA from RSV<sup>3</sup> or avian myeloblastosis virus (AMV)<sup>4</sup>; these template-primer complexes are active in the initiation of DNA synthesis by reverse transcriptase<sup>3-5</sup>.



**Fig. 1** Isolation of 35S RNA-tRNA<sup>Trp</sup> hybrids by sucrose density gradient centrifugation. Prague C RSV 35S RNA (125 µg) was hybridised with <sup>32</sup>P-tRNA<sup>Trp</sup> (26 µg; 70,000 c.p.m. µg<sup>-1</sup>) according to the procedure of Waters *et al.*<sup>4</sup>. The hybridisation mixture was subjected to centrifugation in a 15-30% (w/v) sucrose density gradient as described previously<sup>12</sup>. The radioactivity in each fraction was monitored by Cerenkov radiation and the fractions comprising the high molecular weight RNA region (shaded area) were pooled and precipitated with ethanol<sup>12</sup>. RSV RNA was prepared as described previously<sup>12</sup>. Uniformly labelled <sup>32</sup>P-tRNA<sup>Trp</sup> was isolated by two-dimensional electrophoresis<sup>13</sup> from the free 4S RNA fraction of <sup>32</sup>P-labelled RSV. The identity and purity of tRNA<sup>Trp</sup> was established by fingerprint analysis (see Fig. 3a). Non-radioactive RSV (Prague C) was obtained through the courtesy of Dr R. E. Smith from University Laboratories, Highland Park, New Jersey, under the auspices of the Office of Program Resources and Logistics, National Cancer Institute.



**Fig. 2** Autoradiogram of the polyacrylamide gel fractionation of a 35S RNA-tRNA<sup>Trp</sup> hybrid RNase T<sub>1</sub> digest. P, Position of undigested tRNA<sup>Trp</sup> determined from a parallel electrophoretic run; B, position of bromophenol blue tracking dye; O, origin. Electrophoresis was from top to bottom. RNA hybrids, prepared as described in the legend of Fig. 1, were dissolved in 100  $\mu$ l of 0.01 M Tris-HCl, pH 7.5-0.1 M NaCl. RNase T<sub>1</sub> (Sankyo Co., Japan; obtained from Calbiochem Corp.) was added to give a final concentration of 0.83 U per 100  $\mu$ g RNA. After 30 min at 37 °C, the digest was rapidly cooled, diluted with two volumes of electrophoresis buffer (0.045 M Tris, 1.4 mM EDTA, 0.045 M boric acid, pH 8.3) and subjected to electrophoresis in a 10% polyacrylamide gel<sup>13</sup>. Those areas of the gel containing <sup>32</sup>P-RNA were excised and the RNA eluted as described previously<sup>11</sup>. In the case of small oligonucleotides (area 3), the gel was crushed and suspended in electrophoresis buffer. The radioactive material was then recovered by electrophoresis on to DEAE paper<sup>7</sup>.

We have examined the nature and extent of the interaction between tRNA<sup>Trp</sup> and RSV 35S RNA by characterising fragments of tRNA<sup>Trp</sup> which result from limited digestion of 35S RNA-tRNA<sup>Trp</sup> hybrids with RNase T<sub>1</sub>. The large, undigested fragments of tRNA<sup>Trp</sup> represent regions of the polynucleotide chain which are complementary to the 35S RNA. Earlier studies on bacteriophage RNAs have shown that, in the appropriate conditions, guanylate residues normally susceptible to the action of RNase T<sub>1</sub> are resistant to digestion when base paired in regions of secondary structure<sup>6-8</sup>.

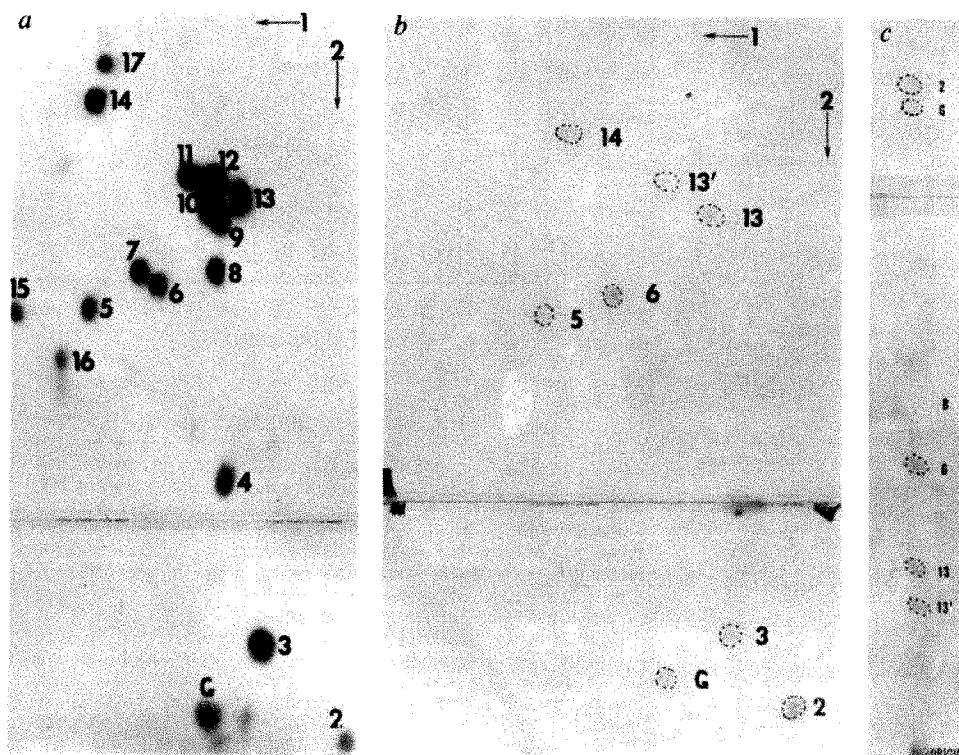
<sup>32</sup>P-tRNA<sup>Trp</sup> was hybridised to non-radioactive 35S RNA. The resulting hybrids were separated from uncomplexed tRNA<sup>Trp</sup> by sucrose density gradient centrifugation (Fig. 1). After recovery by ethanol precipitation, the hybrids were dissolved in a buffer of high ionic strength to stabilise the interaction between the polynucleotide chains, then digested with RNase T<sub>1</sub>. The digestion products were fractionated by polyacrylamide gel electrophoresis (Fig. 2).

Digestion of the 35S RNA-tRNA<sup>Trp</sup> complex resulted in the release of two components which moved more slowly than free, undigested tRNA<sup>Trp</sup> (bands 1' and 1, Fig. 2) and smaller components which had mobilities greater than tRNA<sup>Trp</sup> (band 3, Fig. 2). In some digests an additional component was often present; although not apparent in the digest represented in Fig. 2, its position of migration is indicated as band 2. When unhybridised tRNA<sup>Trp</sup> was digested in the presence of 35S RNA using the same digestion conditions used for the 35S RNA-tRNA<sup>Trp</sup> complex, radioactive material was detectable in the position of band 3, but not in the positions of bands 1', 1 or 2.

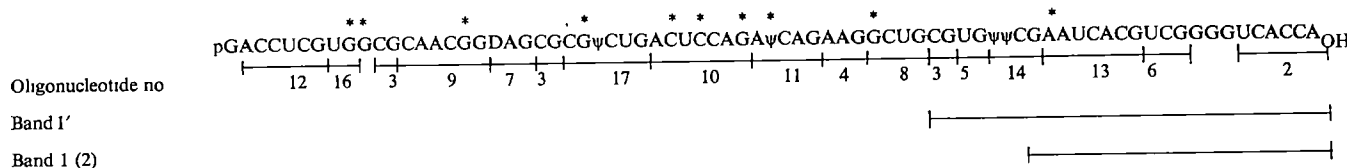
The radioactive fragments comprising bands 1', 1 and 2 were completely digested with RNase T<sub>1</sub> and the products of digestion were fractionated either by one- or two-dimensional electrophoresis. Figure 3 shows the fractionation patterns obtained for bands 1', 1, and tRNA<sup>Trp</sup>. The fractionation pattern obtained for band 2 was identical to that of band 1; band 2 presumably results from partial dissociation of the double-stranded RNA complex (band 1) during dilution in the electrophoresis buffer before electrophoresis.

The oligonucleotides resolved by electrophoresis were identified by comparing their pancreatic RNase digestion products with the pancreatic RNase digestion products of the oligonucleotides obtained from the complete RNase T<sub>1</sub> digestion of intact tRNA<sup>Trp</sup>.

Since the nucleotide sequence of tRNA<sup>Trp</sup> has been determined<sup>1</sup>, it is possible to determine from which region of the tRNA the oligonucleotides released by RNase T<sub>1</sub> were derived. All the oligonucleotides present in bands 1', 1, and 2 are derived from the 3' end of the molecule (Fig. 4). This region of the tRNA must therefore be complementary to the 35S RNA. The analysis of material in the area of the



**Fig. 3** Electrophoretic separation of complete RNase T<sub>1</sub> digests of fragments of 35S RNA-tRNA<sup>Trp</sup> hybrid complexes. *a* and *b*, Fractionation<sup>14,15</sup> of digests of tRNA<sup>Trp</sup> (control) and band 1', respectively. 1, Direction of electrophoresis in the first dimension; 2, direction of electrophoresis in the second dimension. *c*, One-dimensional fractionation on DEAE paper of a digest of band 1. B, Position of the xylene cyanol blue marker; G, guanosine 3'-phosphate. The oligonucleotide numbering system is identical to the system of Faras *et al.*<sup>1,16</sup>. Oligonucleotide 13' results from the rearrangement of m<sup>3</sup>A to m<sup>6</sup>A in oligonucleotide 13.



**Fig. 4** tRNA<sup>Trp</sup> fragments resistant to RNase T<sub>1</sub> digestion in 35S RNA-tRNA<sup>Trp</sup> complexes. The nucleotide sequence of tRNA<sup>Trp</sup> and the numbering system of the oligonucleotides have been described previously<sup>1,16</sup>. \*, Modified nucleotides. Solid lines under a nucleotide sequence indicate regions protected from digestion with RNase T<sub>1</sub>.

gel designated as band 3 showed only the presence of oligonucleotides derived from the 5' end of the tRNA (data not shown).

The finding that the 3' end of the tRNA<sup>Trp</sup> molecule is complementary to the 35S RNA is not surprising in view of the fact that polymerisation by reverse transcriptase results in the covalent linkage between the terminal 3'-OH adenosine of the primer RNA and the first deoxyadenosine residue of the nascent DNA<sup>9-11</sup>.

It seems likely that the interaction of tRNA<sup>Trp</sup> with viral RNA would still allow for the formation of both the anticodon and dihydro U loops as well as the maintenance of the anticodon arm portion of the 'L'-shaped tertiary conformation<sup>17</sup>. In addition, the 3' end of the tRNA may interact with the viral RNA so as to maintain the overall symmetry of the tRNA. The symmetry of the complex might be important with respect to its recognition by reverse transcriptase<sup>18</sup>.

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## 11-*cis* vitamin A in dark-adapted rod outer segments is a probable source of prosthetic groups for rhodopsin biosynthesis

THROUGHOUT life, the outer segments of rod photoreceptors (ROS) are renewed continually<sup>1</sup>, a process that entails

assembly of new disk membranes at the base of the ROS. Biosynthesis of the apoprotein of rhodopsin, the major membrane protein of the ROS disks, is known to occur in several stages in the inner segment<sup>2-5</sup>. Bok has found, however, that completion of the rhodopsin molecule by combination with its retinaldehyde prosthetic group does not take place until after the apoprotein has been transported to the ROS<sup>6</sup>, perhaps just before its insertion in to the membrane. The process continues unimpaired in darkness<sup>7,8</sup>, so that in these conditions a pool of 11-*cis* vitamin A should be readily available close to the site of synthesis.

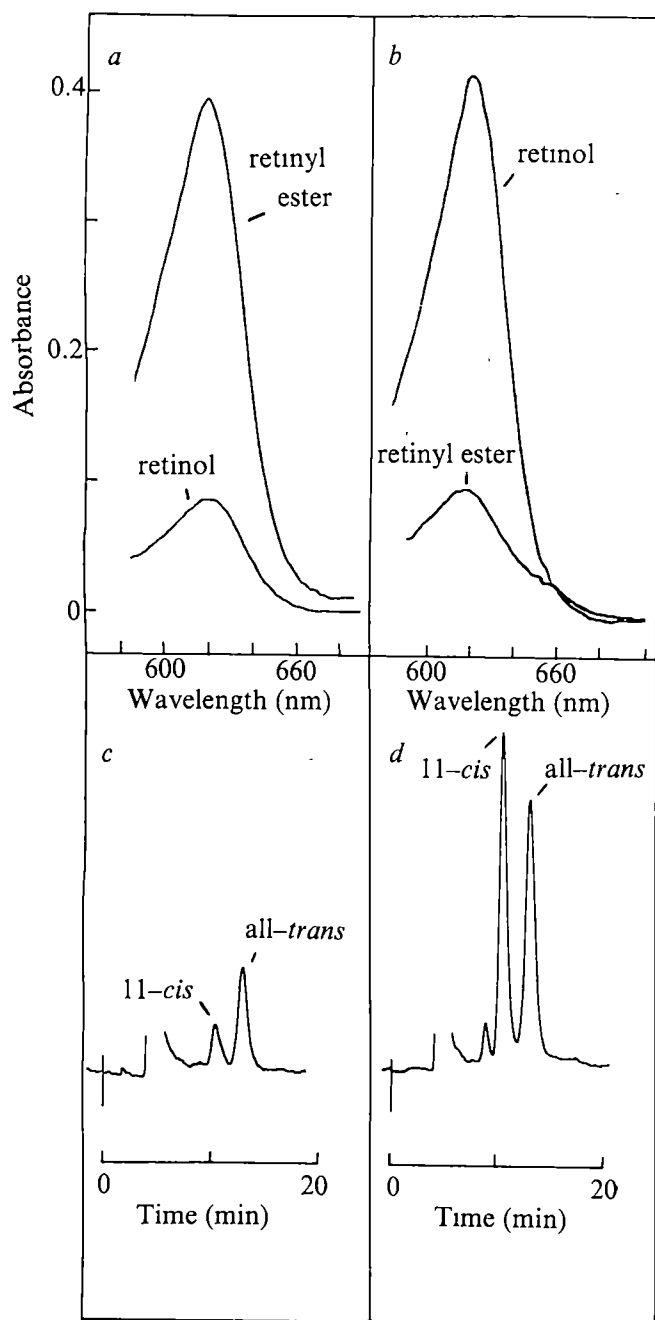
Frogs (*Rana pipiens*, southern variety) were dark adapted for 24 h at 25 °C. The retinæ were cleanly dissected in Ringer solution, any small areas with adhering pigment epithelium being cut away and discarded. Wherever possible, all subsequent operations were carried out in a nitrogen atmosphere with nitrogen-purged solvents. The retinal material was separated into two fractions by syringing through a No. 17 trochar to shear off the ROS and centrifuging on a discontinuous sucrose gradient according to Papermaster and Dreyer<sup>9</sup>. The ROS were quantitatively collected and pelleted in water. Attempts at further purification always resulted in appreciable loss of vitamin A. The residual ROS-denuded retina was washed with water. Subsequent rhodopsin extractions with 2% digitonin indicated that 90% of the ROS had been removed by this procedure.

The tissue fractions were freeze-dried and powdered thoroughly, and the vitamin A extracted by grinding three times with aliquots of light petroleum (b.p. 37-48 °C). A further small quantity of vitamin A could be extracted if the residues were mixed with water, methanol and then light petroleum, but it amounted to only 8-10% of the total. The methanol treatment destroys rhodopsin, however, so appreciable amounts of retinaldehyde were also present.

It was found that the ROS contained a small, but measurable, supply of vitamin A and that it differed markedly in both isomeric configuration and degree of esterification from that found elsewhere in the retina. After filtration (Millipore FHLPO 1300) and concentration to equal volumes, the light petroleum extracts were chromatographed by high pressure liquid chromatography (HPLC; see legend to Fig. 1 for details)<sup>10</sup>. Retinyl esters eluted first and were followed several minutes later by retinol isomers. The separated ester and alcohol fractions were quantitatively transferred to 0.1 ml of dry chloroform. One drop of acetic anhydride was added, followed by 0.9 ml of Carr-Price reagent (saturated solution of SbCl<sub>3</sub> in chloroform). Spectra of the blue colour generated by this reaction are shown in Fig. 1a and b. The  $\lambda_{\max}$  are at 620 nm, and the absorbance depends on the amount of vitamin A present, irrespective of its isomeric configuration and whether it is esterified or not<sup>11</sup>.

The ROS-denuded retina accounted for about half of the total vitamin A, most of it (82%) being in the form of ester. On the other hand, the alcohol predominated (82%) in the ROS, which distinguished them not only from the rest of the retina but also from the pigment epithelium<sup>10,12,13</sup>. The vitamin A in this tissue is almost entirely esterified in the yellow oil droplets and 92% in the cytoplasm<sup>14</sup>.





**Fig. 1** Vitamin A content of ROS compared with the rest of the retina. *a* and *b* show the spectra of the Carr-Price "blues" (Cary 14 spectrophotometer) from the retinyl ester and retinol fractions separated by HPLC as summarised below. Both colour intensity and  $\lambda_{\max}$  are independent of isomer configuration and whether alcohol or ester forms are involved<sup>11</sup>. The ROS contained 3.69 nmol vitamin A of which only 0.69 nmol was ester. From the rest of the retina the yield was 3.48 nmol, of which 2.86 was ester. *c* and *d* show the HPLC records obtained. Retinyl esters eluted between 3.5 and 7.0 min. Other absorbing substances, including small amounts of carotenoid esters, appeared during this time but did not interfere with the Carr-Price reaction. For clarity, the initial peak of the HPLC record has been blocked out. The 11-*cis* and all-*trans* retinols eluted between 9.5 and 16.0 min. The minor peak preceding 11-*cis* in the ROS extract was not identified; its size varied between different experiments. The experimental conditions were: Columns, 2 m  $\times$  2 mm stainless steel packed with Corasil II (Waters Associates), eluant, 1% isopropanol in hexane (Fisher H-291), flow rate, 0.9 ml min<sup>-1</sup> from a Waters model 6000 pump; detection, 310 nm at absorbance 0.05 setting on an Instrument Specialties model UA-5 monitor; injection, 24  $\mu$ l, Chromatronic fixed-loop valve. Recovery of retinol from the column averaged 95%, retinyl ester 100%. *a*, *c*, Retina without ROS; *b*, *d*, ROS.

As Fig 1*b* and *c* shows, the alcohol fractions eluted in two bands maximal at 10.6 and 13.0 min identified respectively as 11-*cis* and all-*trans* retinol<sup>10</sup>. The 11-*cis* isomer accounted for as much as 60% of the ROS retinol, but the all-*trans* isomer preponderated in the small quantity of retinol present in the remainder of the retina.

The vitamin A content of the ROS amounts to 4.2 mol % of the rhodopsin, determined by digitonin extraction of the light petroleum-extracted residues. This corresponds to about a 2-d supply of prosthetic groups if ROS growth proceeds at the rate of  $\sim 2\% \text{ d}^{-1}$  (ref. 1). The amount of 11-*cis* retinol alone is thus sufficient to sustain rhodopsin renewal for about 1 d. Normally, the pool of ROS retinol would be continually replenished, probably from supplies in the pigment epithelium which are known to amount to about 2 mol of retinyl ester per mol of retinal rhodopsin<sup>10</sup>. The ester could be converted to alcohol before transfer to the retina, but it is more likely that it is transported unchanged and hydrolysed in the ROS. The latter cannot synthesise ester, although other parts of the retina can<sup>14-16</sup>. The derivative of vitamin A that is converted to 11-*cis*, and where this isomerisation takes place in the eye, is being investigated and will be discussed elsewhere.

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## Corrigendum

In the article "Mutation rate, genome size and their relation to the rec concept" by J. A. Heddle and K. Athanasiou (*Nature*, **258**, 359, 1975) the following corrections should be made. Page 360, line 5: the value of *m* should be  $1.68 \times 10^{-19}$ ; line 6: the *b* and *r* values should be 0.91 and 0.98, respectively. In Table 2 the genome size of  $\phi$ X174 and  $\phi$ 13 should be  $1.7 \times 10^6$ .

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# matters arising

## Chinese cosmology

GRIBBIN'S essay<sup>1</sup> is based on a few pages of Joseph Needham's *Science and Civilisation in China*<sup>2</sup>, but makes an exaggerated claim for Chinese openmindedness as contrasted with Western narrowness by disregarding Needham's sensitivity to context and implications, and by painting an extremely one-sided picture of the European tradition. The statement that Western science was a branch of religion three hundred years ago is either misinformed or extremely ill-stated. Gribbin confuses cosmology and astronomy when he talks about the centre of development of the science shifting to the West by the end of the seventeenth century. "The science" must mean astronomy, mentioned in the preceding sentence. It is impossible to sustain an argument that the centre of astronomy lay in China until that time. Needham proves that early Chinese astronomy was as estimable as that of the West, but his argument ignores the consistent superiority of Europe in computational astronomy. Gribbin is apparently unaware of the work of Nakayama<sup>3</sup>, myself<sup>4</sup>, and others who have provided independent quantitative comparisons in the nearly twenty years since volume 3 of *Science and Civilisation in China* was completed.

Gribbin's article reads like a caricature of Needham, because he ignores everything that does not fit his thesis. One could make an equally one-sided argument in the other direction by noting that Aristarchus of Samos, in the third century BC, asserted that the Earth revolves about its own axis and about the Sun, an idea that never appeared independently in China. Aristarchus also suggested that the stars were immeasurably far from the Sun, but Gribbin does not mention this.

Although it is true, as Gribbin says, that any study of Chinese astrology and cosmology must draw on Needham's work, it cannot end there. Needham's sections on astronomy and mathematics depend much more heavily on the secondary literature than his later volumes. His accounts of early cosmology require correction from other sources. The earliest concept did not, as Needham and Gribbin claim, represent the heavens as a hemispherical dome. As Nakayama has

shown in English, there were two stages; in the first sky and Earth were flat, and in the second they were indefinitely vault-shaped. The second concept was not that of "a celestial sphere surrounding the spherical Earth". Recent Chinese research has proved what Western writers have suggested, namely that the Earth in this model was either flat or hemispherical<sup>5</sup>. Neither concept, in fact, was a cosmology either in the modern sense or in the sense that gave philosophical cosmology authority over computational astronomy in the early West. The two Chinese concepts did not displace each other because it was a matter of no practical consequence which was correct. The independence of astronomy from cosmology in China has been noted by many recent writers. Of the several ancient authorities that Needham cites regarding the "empty space" theory, only one was an astronomer.

Gribbin's reading on supernovae also inadequately represents the very considerable literature that has accumulated<sup>6</sup>. He does not mention the best-documented supernova of all, that of 1006, and does not seem to be aware that Ho *et al.* have produced evidence to cast considerable doubt on his identification of the supernova of 1054 with the Crab Nebula<sup>7</sup>.

Now let me take up what I consider ill advised about Gribbin's topic itself. Historians of science have by now given up treating ancient speculations as foreshadowings, forerunners, or anticipations of the precise concepts of the modern exact sciences, since no one who has hunted for such anticipations with sufficient determination (and willingness to abide bad metaphors) has failed to track them back to the dawn of history. Instead historians think of early ideas as constituents in an endlessly complex and never predictable development from one phase of understanding and practice to the next. I am unable, in any case, to understand why Gribbin believes the Mohist idea that motion requires duration is equivalent to any innovation of Einstein. He also finds deep significance in the fact that the Chinese language includes a compound in which one character stands for space (in the everyday sense) and one stands for time, but this is a purely lexical matter, and has nothing at all to do

with the modern physical concept which we express inadequately in quotidian language as "a continuum in four dimensions".

To sum up, Dr Gribbin has not taken the trouble that he assuredly would have done had he prepared a scientific paper for *Nature*. He has not studied enough, and has not taken sufficient pains to understand what he has studied.

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## Moinian and Lewisian of Sutherland and the Morarian Orogeny

IN view of speculation concerning the Moinian geology of Sutherland<sup>1,2</sup>, a short resume of our research in central Sutherland (S.J.M.) and the Bettyhill-Strathly area of north-eastern Sutherland (V.E.H.) is of interest.

In central Sutherland there are three large, sub-parallel Lewisian sheets within the Moinian west of the migmatite complex<sup>3</sup>. In the Bettyhill-Strathly area there are sheets and broad areas of Lewisian. Our extensive geochemical data indicate that the majority of the Lewisian in these areas has strong Scourian affinities<sup>4-6</sup>. The Borgie Lewisian<sup>6</sup> and that at Ribigill near Tongue (unpublished), contains both Scourian and Laxfordian types. Geochemical distinctions between the western and the eastern Moinian in northern Sutherland<sup>7</sup>, cited by Garson and Plant<sup>1</sup>, represent a distinction between Moinian with extensive basic Lewisian in the west, the Tongue-Borgie area, and acid Lewisian with

some Moinian in the east, the Armadale-Strathly area.

Garson and Plant<sup>1</sup> and Johnson<sup>2</sup> mention ultrabasic bodies intruding the Moinian and the Lewisian of Sutherland, as originally described by Read<sup>3</sup>. Our detailed field mapping has shown them to be restricted to the Lewisian. Read<sup>3</sup> and the later authors<sup>1,2</sup> apparently confuse a series of Lewisian metaperidotites and garnet pyroxene rocks (garnet pyroclastics) with early Moinian metabasic intrusives. Geochemical and petrological evidence indicates that the garnet pyroxene rocks of the Naver and Borgie<sup>4</sup> Lewisian are identical to those of the foreland Lewisian<sup>9,10</sup>. Similarly, the metaperidotites are identical to analogous rocks found throughout the foreland Lewisian<sup>8,10,11</sup>. These bodies are not, therefore, synchronous with the emplacement of the Lewisian sheets (see ref. 2), they are an integral part of them. Neither are they high level diapirs (see ref. 2); nor do they constitute a Moinian ophiolite zone (see ref. 1).

Several authors have speculated an extension of the Sgurr Beag Slide<sup>12</sup> into central Sutherland<sup>2,13,14</sup>. Although slides are present there is no definite evidence that any are continuous with the Sgurr Beag Slide. The Lewisian sheets of central Sutherland seem to have been emplaced by a combination of early (F1) isoclinal folding and possible sliding, which has been modified by later (F2) folding and sliding. The continuation of the Sgurr Beag Slide may, however, occur in north-eastern Sutherland, thus extending Johnson's speculation<sup>2</sup>, the Lewisian of central and northern Sutherland may be the root zone of Moravian nappes represented by the large areas of Lewisian in north-eastern Sutherland.

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## Evolutionary changes in insulin

BLUNDELL and Wood<sup>1</sup> propose "a model for the evolution of insulin mainly in terms of adaptive processes". Simultaneously they raise questions regarding "conclusions made on the basis of uniform rates and changes that seem not to affect function", such conclusions being attributed to Kimura<sup>2</sup> and to King and Jukes<sup>3</sup>. They do not mention the more recent discussion by Kimura and Ohta<sup>4</sup>, who point out that insulin in general has a very low evolutionary rate, corresponding to a strong negative-selection barrier. Blundell and Wood's model calls for functional restraints on most of the amino acid residues. Kimura and Ohta also noted that the C peptide of proinsulin evolves about 10 times as fast as insulin, and is therefore a much better candidate for neutral mutations than insulin itself. and they proposed that the absence of zinc from guinea pig insulin results in a loss of constraint on the fixation of mutations.

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## Specificity of transfer factor

In an article on the use of transfer factor (TF) in treatment of viral infections<sup>1</sup>, Zuckerman states that "clinically, TF activates uncommitted and non-sensitized lymphocytes of the recipient so that new clones of antigen-specific cells are produced". He goes on to make the point that the delayed-type skin reactivities that are transferred after injection of TF are those possessed by the donor. It is undoubtedly true that the results obtained during the past 25 yr by Lawrence and others are most readily explained by the existence of such specificity<sup>2</sup>. The problem is, however, that nobody has found a way in which specificity can be demonstrated in unequivocal terms and by procedures which allow for confirmation in other laboratories. At the recent Second International Workshop on Basic Properties and Clinical Applications of Transfer Factor at Fort Detrick, there were more than 100 participants and not one convincing demonstration of specificity.

Failure to convince does not disprove the concept of antigen specificity, but it is worrying. It may be due to the difficulties of human experimentation and the lack of a satisfactory animal

model. One possibility is that TF preparations contain both a specific factor and nonspecific adjuvant-like factors, and that this is creating problems in man where the antigens normally studied are not complete strangers to the 'negative' donors and recipients. The various *in vitro* systems that have been used have not pointed to specificity, although there is no guarantee that the factors active *in vivo* are being assayed. The work of one of us has involved the action of human TF on macrophage migration and lymphocyte transformation<sup>3,4</sup>. Evidence obtained in the latter system suggests that the action of TF is nonspecific but that the level of this nonspecific activity is greater in tuberculin-positive than in negative individuals.

Doubts about specificity are especially relevant in the clinical field where there is clear evidence that TF can boost phytohaemagglutinin responsiveness and the number of cells forming rosettes with sheep erythrocytes<sup>5,6</sup>. This nonspecific action could be responsible for the appearance of delayed hypersensitivity and clinical improvement.

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## Insectivorous grouse?

BEFORE embarking on the "full-scale investigation of the relationship between grouse and insects" recommended by your Animal Ecology Correspondent<sup>1</sup>, the cautious research worker might wish to establish that incubating hen red grouse do in fact eat many tipulids. This is crucial to the suggestion that hen grouse on wet moors may rely on tipulids to redress their supposed state of 'nutrient depletion' after egg laying. The work by Butterfield and Coulson<sup>2</sup> did not show that adult hen grouse eat tipulids at this stage, although it would not be surprising if readers of their paper were to infer that they do.

Although Butterfield and Coulson present data for the month of May, when hens are incubating, the dropings that they collected in May were from cock grouse. How do we know this? When incubating, hen grouse

produce large, distinctive 'clocker' droppings, rather like those of broody chickens. Before their paper was submitted, we asked Dr Coulson if clocker droppings had been included in the May sample, and we also described what they look like. He said they had noticed some droppings like that, but discarded them as they did not know what the droppings were.

If tipulids do 'play a vital part in the maintenance of grouse populations', as suggested by your Animal Ecology Correspondent we should expect wet, blanket-bog grouse moors where there are many tipulids to support more grouse than drier moors. In fact, the reverse is the case. The wettest moors have the fewest grouse. Contrast western Ireland where the extensive, wet bogland supports good populations of tipulids (A. N. Lance, personal communication) but very few grouse<sup>3</sup> with the typically dry moors in the north-east of Scotland where grouse are abundant<sup>4</sup>. Almost all good grouse moors are on dry ground and the association between dry ground and good grouse shooting is so widely understood that draining wet ground to increase grouse numbers is an accepted management practice.

The reason for this is clear. Heather covers a greater proportion of the ground and grows best on dry moors, and numbers of grouse are correlated both with the proportion of ground covered by heather and with the vigour of its growth<sup>5</sup>.

The nutritional requirements of an adult grouse are highest when the hen is laying eggs. It is this time when nutrients are most likely to be limiting and when insect food is most likely to be an important dietary supplement. Laying hen grouse do not eat many insects, because insects are not abundant this early in the season. The suggestion remains that hens become depleted of nutrients while laying eggs and that insect food, especially tipulids, is essential during the incubation period in order to redress this hypothetical imbalance. It is not claimed that this occurs on dry moors, because adult grouse eat few insects on dry moors. The suggestion is limited to wet moors.

This limitation implies that there must be some difference between the diets of laying hens on wet and dry moors, such that hens on wet moors are less well fed than on dry moors. This is possible because one important determinant of the hens' plane of nutrition in spring is the density of heather available<sup>6</sup>; and heather tends to be sparser on wet moors<sup>7</sup>. Therefore we agree that it would be wise to improve the hens' plane of nutrition in spring.

It is most helpful to improve the

food for the hens before they begin to lay eggs. When the laying hens' food is good, the chicks which hatch survive better<sup>8</sup> as a result of improvement in the quality of the eggs<sup>9</sup>. The food can be improved by proper heather management, which includes draining on wet moors and results in the provision of a dense array of heather shoots. The alternative suggestion, mooted by your Animal Ecology Correspondent, is that tipulids should be provided after the hen has laid her eggs. This procedure can have no effect on the quality of the eggs.

There is no evidence that hen grouse do eat tipulids during incubation. Even if they do, there is no evidence that tipulids are ever in short supply on wet moors. The only observed association between tipulid densities and grouse numbers is that grouse stocks are higher where there are fewer tipulids—on dry moors.

We do not attempt to argue that tipulids are totally unimportant on wet moors, because there is no direct evidence one way or the other. But we do suggest that heather will remain much more important for grouse numbers than tipulids, even if the 'nutrient depletion' hypothesis proves correct.

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## Temperature, phenoxybenzamine and adrenoceptor transformation

In his letter<sup>1</sup> Benfey suggests that the adrenoceptor transformation hypothesis is incorrect because temperature-induced changes in the block by and binding of phenoxybenzamine (POB) in frog hearts<sup>2</sup> could result from a change in the rate of alkylation of nonspecific nucleophilic centres. The impression is given that the hypothesis is based only on the observed effects of POB, however, several reports indicate that the competitive  $\alpha$ -adrenoceptor antagonist, phentolamine, can also effectively inhibit inotropic and chronotropic responses to catecholamines in the hearts of various species at low temperature<sup>3-7</sup>. In addition, tem-

perature is not the only factor that has been shown to alter the properties of adrenoceptors. Cardiac inotropic and chronotropic responses in hypothyroid rats are affected by POB and phentolamine at high temperatures similarly to those of normal hearts at low temperatures<sup>8-10</sup>. These observations clearly cannot be due to an effect of temperature on nonspecific alkylation. A nonspecific temperature effect might explain Benfey's observation<sup>1</sup> that POB blocks acetylcholine more effectively at higher temperatures. This temperature effect, however, is opposite to that observed with cardiac adrenoceptors, and parallels the increase in POB binding we have found in homogenised myocardium. Protection from POB block by high concentrations of membrane stabilisers does not prove that  $\alpha$ -adrenoceptors are not involved. Local anaesthetics can influence the apparent affinity of an antagonist with  $\alpha$ -adrenoceptors<sup>11</sup>.

The concentration of POB required to block responses to adrenaline in frog hearts at low temperature is clearly higher than that required to block  $\alpha$ -adrenoceptors in vascular smooth muscle, however,  $\alpha$ -adrenoceptors are probably not all identical. Relatively high (micromolar) concentrations of either POB or phentolamine are necessary to inhibit presynaptic  $\alpha$ -receptors in the heart<sup>12,13</sup> and all but one study demonstrating an  $\alpha$ -adrenoceptor component of inotropic responses of mammalian hearts have used high concentrations of  $\alpha$ -blocking drugs<sup>8,10,14-17</sup>. The only claim that nanomolar concentrations of POB or phentolamine can inhibit responses to phenylephrine in rabbit left atria<sup>18</sup> is disputed<sup>19</sup>. Lack of complete block with even high concentrations of  $\alpha$ -adrenoceptor antagonists could also reflect the fact that no 'pure' cardiac  $\alpha$ -adrenoceptor agonist is available, and the considerable residual  $\beta$  activity at low temperature, or in hypothyroidism, could 'mask'  $\alpha$ -adrenoceptor block.

The absence of a temperature-induced difference in the blocking action of propranolol or in the relative potencies of agonists reported by Benfey is contrary to observations from several laboratories<sup>3-7</sup>, and the reason for the difference is not clear. His data do, however, suggest that propranolol is more effective in hearts that are more responsive to  $\beta$ -adrenoceptor stimulation. The standard errors in his Table 1 indicate up to 50-fold variation in sensitivity to isoproterenol. Propranolol greatly reduced this variability, showing a greater effect on the more sensitive preparations. Benfey does not indicate the temperature during exposure to propranolol: incubation at 24 °C before testing the block at 14 °C could account for the lack of a decrease in inhibition



It is unlikely that the previously reported effect of propranolol at low temperature<sup>3-7</sup> was due to inadequate diffusion of the drug. Benfey found that atropine produced the same block at low and high temperatures after 10-min incubation. Hypothyroidism also reduced the inhibitory effect of propranolol, although all tests were at the same temperature and after 30-40-min incubation<sup>8-10</sup>.

In conclusion, Benfey's data seem to be inadequate to support his contention that the hypothesis of adrenoceptor transformation is an artefact attributable to nonspecific alkylation by POB

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<sup>1</sup> Benfey, B. G., *Nature*, 256, 745 (1975)

<sup>2</sup> Kunos, G., Yong, M. S., and Nickerson, M., *Nature new Biol.*, 241, 119 (1973).

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<sup>4</sup> Buckley, G. A., and Jordan, C. C., *Br. J. Pharmac.*, 38, 394 (1970).

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<sup>18</sup> Benfey, B. G., *Br. J. Pharmac.*, 48, 132 (1973).

<sup>19</sup> Wagner, J., and Reinhardt, D., *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmac.*, 282, 292 (1974).

**BENFEY REPLIES**—The above comment<sup>1</sup> on my letter<sup>2</sup> misses the point. The real issue is not whether a change of temperature alters the rate of alkylation by phenoxybenzamine (although it obviously does), it is not how much phenoxybenzamine is required to block  $\alpha$ -adrenoceptors (although Kunos *et al.*<sup>3</sup> loaded the tissue by four 10-min exposures to 2.5  $\mu$ g ml<sup>-1</sup> of the highly lipid-soluble drug), and it has nothing to do with hypothyroid rats (although it may be noted that mammals have cardiac  $\alpha$ -adrenoceptors in normal conditions at normal temperature<sup>4</sup>). The issue is whether lowering temperature transforms the adrenoceptor of the frog heart from its characteristic  $\beta$ -type into an  $\alpha$ -receptor. If this were so, one might reasonably expect that phenylephrine would be more potent at a low than at a high temperature,

that the reverse would be true for isoprenaline, and that the blocking ability of propranolol might be changed by lowering temperature. These predictions were not supported by my experiments, and this was the reason for not accepting the hypothesis that the nature of the adrenoceptor changes with temperature<sup>2</sup>.

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<sup>1</sup> Kunos, G., and Nickerson, M., *Nature*, 259, 251-252 (1976).

<sup>2</sup> Benfey, B. G., *Nature*, 256, 745 (1975).

<sup>3</sup> Kunos, G., Yong, M. S., and Nickerson, M., *Nature new Biol.*, 241, 119 (1973).

<sup>4</sup> Benfey, B. G., *Br. J. Pharmac.*, 48, 132 (1973).

## *Aedes aegypti* complex in Africa

We wish to discuss aspects of the communication by Scott and McClelland<sup>1</sup>. It was pointed out long ago that sound ecological studies depend on the accurate determination of the evolutionary status of the organisms under study<sup>2</sup>. This is especially crucial in applied biology<sup>3</sup>. If two forms are common and sympatric there are three possible situations. (1) They are reproductively isolated and do not exchange genes under natural conditions; in other words they are distinct species. If they are not reproductively isolated (that is they mate with each other in nature), they may represent (2) two subspecies (races) whose ranges have recently intersected to form a transient area of sympatry where hybridisation occurs, or (3) morphs of a single polymorphic species<sup>4</sup>. The concepts behind these possibilities are well established and backed by sound bodies of theory, and many studied examples of each are documented<sup>5</sup>.

Scott and McClelland dealt with the sympatric occurrence of the dark and pale forms of the current taxon *Aedes aegypti* L., a mosquito of considerable medical importance. The pale form has a wide distribution within and beyond Africa, and is domestic in its habits. The dark form is confined to Africa south of the Sahara, and is an outdoor mosquito<sup>6</sup>. These facts and the data on the alkaline phosphatase and protein loci provided in Table 1, of ref. 1, are sufficient to eliminate the possibility that we are dealing with a single polymorphic species. In fact, the data for these two loci provide strong evidence for the existence of positive assortative mating in the field, thus supporting the view that they are distinct genetic species. It should be emphasised that the evidence for random mating in cages and for the interfertility of the two forms is of little relevance in assessing the status of the forms because it is well established that distinct genetic species are often interfertile

in artificial crosses<sup>7,8</sup>, and cases are known where distinct species mate at random in cages<sup>7,9</sup> but positively assortatively in nature<sup>7,10,11</sup>.

We think that it is unfortunate that Scott and McClelland used the vague and imprecise terms "ecotype" and "incipient species". "Ecotype" is a term introduced for plants and is not appropriate to animals. The term "incipient species" is not appropriate here, as it is usually used in narration to imply some well marked subspecific stage. Neither term is backed by a satisfactory body of theory. The opening sentence of the report immediately leads to confusion by implying that "ecotypes" are not populations.

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<sup>1</sup> Scott, J. A., and McClelland, G. A. H., *Nature*, 256, 405-406 (1975).

<sup>2</sup> Elton, C. S., *Animal Ecology* (Macmillan, London, 1927).

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**SCOTT AND MCCLELLAND REPLY**—We agree that the status of the two entities is crucial to the paper. We believe that field hybridisation has been amply demonstrated (refs 4 and 9 of our report in *Nature*<sup>1</sup>). A computer simulation of this situation containing three genotypes, three habitats, two seasons, parameters for rate of movement, habitat selection, natural selection and assortative mating, is now under review. It demonstrates that polymorphism is highly probable, provided only that: (1) there is a dry season when breeding occurs only in the indoor habitat; (2) fitness of the indoor ecotype is greater in houses, and fitness of the outdoor ecotype is greater in the natural habitat; and (3) movement between indoor and outdoor habitats is not large (less than about 25% of the indoor inhabitants must be immigrants each generation). The computer model shows that, when mating is random, polymorphism is probable.

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<sup>1</sup> Scott, J. A., and McClelland, G. A. H., *Nature*, 256, 405-406 (1975).

# reviews

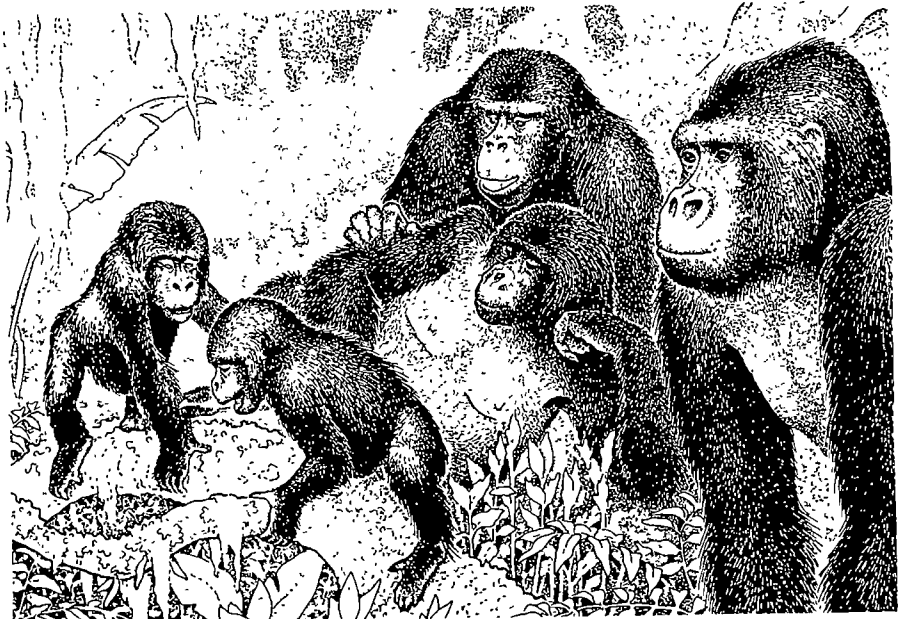
## Bull's-eye of sociality

THIS is a book\* addressed to a wide audience and one likely to make a lasting impact on biological thought, research and teaching. Its title puts a new name to a subject that is among the fastest-growing areas of biology, and its contents do indeed provide a new synthesis, of wide perspective and great authority.

Dr E. O. Wilson is one of the best-known zoologists of the day, and collaborated with the late Robert MacArthur in writing the biological best-seller, *Island Biogeography* (1967). Since then he has produced, among other works, a large, universally acclaimed monograph on *The Insect Societies* (1971). His output is extraordinary: *Sociobiology* itself contains about half a million words. His plain uncluttered prose is a treat to read, his logic is rigorous, his arguments (even the occasional mathematics) are lucid. He is a real polymath, threading a constructive path through forests of zoological literature and, as far as one can tell, rarely putting a foot wrong. In a difficult and controversial field, his deductions are informed, balanced and fair, even though some are necessarily questionable.

The book is an evolutionary study of social biology throughout the animal world, or more exactly, throughout the Metazoa. It is divided into three sections of which the first, 'Social Evolution' is the shortest, and deals among other subjects with the fundamentals of sociality, population biology, and selection processes. The second, *Social Mechanisms*, covers communication, dominance and territorial systems, parental care, and much else. The third is entitled *The Social Species*, and in 200 pages provides a critical review of more than 30 selected groups, chosen from the invertebrates, cold-blooded vertebrates, birds, mammals and primates (including man). The coverage and digestion of the literature are amazing: about 2,500 references are listed, making the bibliography a goldmine in its own right. At £11 the book is a bargain.

\**Sociobiology: The New Synthesis* By Edward O. Wilson. Pp. 697 (Belknap Press of Harvard University: Cambridge, Massachusetts, 1975.) £11.



I am no stranger to the perplexities of sociobiology; they have occupied much of my time for over 20 years. Having read the book through I find myself wondering how close Professor Wilson has come to the bull's-eye, in pondering what sociality is and does, as an evolutionary adaptation. The basic target is not easily perceived, perhaps because the adaptive value of sociality is different, at least superficially, for different animals. The simple definition he gives of a society (p7) is "a group of individuals belonging to the same species and organised in a cooperative manner". He stresses (p379), as being among the key properties of social existence, cohesiveness, altruism and cooperativeness, and often, especially in reviewing the social species, he contrasts social with solitary animals, as if flocking or sociability were also a key property. Thus he affirms (p484) that sociality has been advanced in those African mammals that have forsaken the closed forests and become adapted to open grasslands. But we know that there are many kinds of fish, birds and mammals that vary their flocking strategy from day to day and hour to hour, sometimes in fact depending on whether they are in open or closed habitats. Can the distinction between clumped and solitary dispersion indeed be fundamental?

He sees four pinnacles of social evolution, found in (1) the colonial invertebrates, (2) the social insects, (3) the vertebrates and (4) man himself, and he observes that the first three

have been scaled many times by independently evolving lines of animals, although man has scaled his peak on his own. In the context of Professor Wilson's special group, the social insects, he picks out as their particular social traits, the cooperation of individuals in the care of the young, the presence of more or less sterile individuals working on behalf of fecund nestmates, and an overlap of at least two generations capable of contributing to colony labour.

It is perhaps here that he comes nearest to identifying two other properties that seem to me to lie still closer to the bull's-eye of sociality: first, the potential endurance of animal societies, so that they characteristically outlive their current members; and second, their property of imposing duties and restraints on their members, serving to promote the common good, often in the longer term. Selection for altruism itself is admirably discussed.

He regards those animals, the colonial invertebrates, that have scaled the first pinnacle as coming close to producing perfect societies; and, looking towards the other three pinnacles, he asks why the overall trend has been downward from this primitive or simple perfection. The siphonophores are to him "the *ne plus ultra*"; whereas from my different point of view it is hard to concede that they qualify as societies at all. They are of course composite or compound animals, made up of variously modified polyps, and they use budding as a means of growth and organ differentiation; but each siphono-

phore complex develops from a single zygote into a ciliated larva, and later into a primordial polyp, forming, as it grows, a stem from which the other polyps branch. After a reproductive period its planktonic existence ends, and it dies as an entity. Perhaps it is not in every sense one individual; but can its state of organisation properly be equated with that of the higher societies on any of the other pinnacles—societies composed of separate, potentially competing, genetically different, sentient individuals, bonded together by sensory recognition and appetite, constrained by conventions

and by traditions handed down from forbears to descendants as the generations pass. Once more I must doubt it, and whether indeed the author could claim (which of course he does not) yet to have found the keys to this black box.

Ultimately these questions are important, but for the present I think they will worry most readers not at all, nor detract from the pleasure and profit to be found in this remarkable book. They do nothing to diminish its new horizons, but remind us only that we are not at the end of the road.

V. C. Wynne-Edwards

## Superconductivity

*The Superconducting State.* (Graduate Student Series in Physics.) By A. D. C. Grassie. Pp. vii+135. (For Sussex University Press; Chatto and Windus: London, September 1975.) £8.00.

THE author, in his preface, excuses the appearance of yet another book on superconductivity by claiming that "It corresponds to the level of the experimental physicist who needs to follow theoretical papers in order to analyse his results adequately . . ." Having on many occasions suffered restrictions imposed by a lack of theoretical understanding, I approached this volume with eager anticipation. It is with regret that I report that after reading, and indeed re-reading, the book my hopes remain unfulfilled.

The first chapter, an introduction to the phenomena of superconductivity, sets the historical perspective. The second, dealing with the microscopic Bardeen, Cooper and Schrieffer (BCS) theory of superconductivity requires fairly advanced quantum mechanics, and will severely tax most experimental physicists. A less mathematical treatment of BCS is probably not possible, but a closer attempt to relate formulae to physical reality would be helpful. The strong-coupling extension of BCS, which is needed to discuss the technically interesting, high critical temperature superconductors, is dismissed in two paragraphs. The limitations of the theory, for example in its ability to predict new superconductors, are ignored.

The following two chapters cover the Ginsburg-Landau phenomenological theory, and the magnetic state of type II superconductors. The treatment is on a par with those to be found elsewhere, and offers no new insight. The latter section is incomplete in that it ignores the temperature dependence of  $\kappa$ .

The reader is entitled to expect more technological bias from chapter 5, en-

titled "The Current-Carrying Capacity of type II Superconductors". The main reason for using superconductors in large electrical devices is their ability to sustain very high current densities, but the pinning of flux lines, which is directly responsible for the magnitude of the critical current, receives one paragraph. It is true that "the origin of pinning forces is still a subject of detailed research", but then so are many of the other phenomena described. There is sufficient agreement among workers interested in flux-pinning for the author to have given some indication of the physical nature of pinning forces. The discussion of flux instabilities is better, though in one of the all too rare numerical calculations the author arrives at the incorrect value of 0.042 cm for the critical half-thickness of a stable conductor. Using the author's formulae and quoted values for parameters, the correct answer should be 0.003 cm, a value which looks less out of place with the accompanying photograph of a multifilamentary conductor.

The final chapter is concerned with Josephson effects, and the devices which use them. This is the best chapter in the book, not only because it is the most original. But at six pence a page, the book must be regarded as expensive.

David Dew-Hughes

## Insect biochemistry

*Insect Biochemistry and Function.* Edited by D. J. Candy and B. A. Kilby. Pp. xii+314. (Chapman and Hall: London, May 1975. Distributed in USA by Halsted Press.) £8.50.

THOSE expecting a comprehensive textbook of insect biochemistry will be disappointed. This book is in fact a collection of four review essays describing the biochemistry of certain functions in detail, and the authors assume the reader to have a good basic knowledge of biochemistry. The

greater part of the book is devoted to aspects of insect flight, with one chapter on the utilisation, and another on the sources of fuels for flight. The first describes the metabolic pathways for the release of energy from carbohydrates, fats and amino acids. Emphasis is placed on the complex interactions resulting in the breakdown of muscle glycogen as an immediate source of energy, the importance of certain metabolites (especially  $\alpha$ -glycerol phosphate, proline and acetyl carnitine) for penetrating mitochondria, and the mechanisms responsible for controlling mitochondrial metabolism, which may increase by as much as 100 fold on the initiation of flight. The nature of these fuels for flight, and the sites for their storage are described belatedly in the second chapter. The importance of the various energy sources for both short and long periods of flight are discussed, and attention is drawn to the central role of the fat body in synthesising and storing these fuels.

The diversity and origins of excretory materials produced by insects is the subject of the third essay which dispels the firmly entrenched generalisation that insects are primarily uric acid excreting organisms. Many nitrogenous compounds are excreted, including ammonia which, despite its great toxicity, is now recognised as a major excretory product in some terrestrial insects. The accumulation of uric acid within the insect body has until recently been thought of as excretion, but evidence is reviewed that this metabolite is mobilised in times of dietary stress, possibly by intracellular symbionts within the fat body.

The final contribution is a concise assessment of our current knowledge of synaptic transmission in insects. After a general outline of the structure of the insect nervous system, cholinergic transmission is summarised very briefly, and the remainder of the chapter is devoted to a discussion of the evidence for glutamic acid,  $\gamma$ -amino butyric acid and biogenic amines as excitatory and inhibitory transmitters both peripherally and in the central nervous system.

Although the editors draw attention to the importance of comparative aspects of biochemistry in achieving selective toxicity in chemicals used for pest control, little emphasis is placed on these, even in the most relevant chapter on synaptic transmission. The book provides, however, good reviews of the three topics, and these have comprehensive bibliographies (the chapter on excretion being especially notable in this respect). It should prove useful reading for both undergraduates and research workers in insect biochemistry. A. L. Devonshire

## Tool not a discipline

*Enzyme Kinetics: Behaviour and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems.* By Irwin H. Segal. Pp xxii+957. (Wiley-Interscience: New York and London, July 1975.) £15.00.

ONE might have expected a book on enzyme kinetics by I. H. Segal to make as useful a contribution to its field as his *Biochemical Calculations* has made within its own category of elementary textbooks. Unfortunately, this has proved otherwise. His new volume is a long and detailed account of just one aspect of enzyme kinetics: steady state methods. Yet it is possible to read through its excess of 900 pages and at the end of it not to have learnt anything about the behaviour of enzymes. The rationale of the study of the kinetics of enzyme reactions is surely the information that kinetic methods provide about the operation of enzymes, either from the viewpoint of the elucidation of the chemical mechanism of the enzyme or the understanding of some biological phenomena. In my opinion, enzyme kinetics is a tool of biochemical research, not an intellectual discipline within its own right. This book presents the latter view.

The heart of the book is a catalogue of steady-state rate equations for enzyme mechanism containing one, two or three substrates reacting with the enzyme in all possible sequences together with the inhibition patterns expected for different types of inhibitors. The mathematics have been presented clearly and double-reciprocal plots of reaction velocity against reactant concentration illustrate most of the equations. No examples of the use of these equations in enzymology are given, which is regrettable because the value of a method can only be judged by the problems it solves. The nomenclature of enzyme mechanisms used throughout the book is that of Cleland. But the principles of steady state kinetics were established more than 20 years ago by Haldane, Alberty and Dalziel, who demonstrated when these methods could distinguish between alternative reaction mechanisms and, equally important when they could not. The newer nomenclature of Cleland is useful on account of its systematic approach but yields no new principles nor unifying concepts. The same comment might apply to this book. The effort here has been expended on description rather than comprehension. For instance, it has never seemed to me helpful to use terms such as Bi Uni Uni Ping Pong Ter Bi to denote a reaction mechanism since they fail to advance an understanding of the mechanism at the physical level.

Therefore, this book is recommended only to professionals working in the field of steady state enzyme kinetics. Though the derivation of the rate equations for any single mechanism is tedious rather than difficult, some will find it useful to have available a compendium of steady state equations for a variety of different systems derived for them. But the restriction to a single experimental method treated in a purely theoretical manner renders the book unsuitable for the general student of biochemistry. One can admire the rigour with which the author approaches his subject but not the basic premise from which he starts.

S. E. Halford

## Breath of air

*Principles of Comparative Respiratory Physiology.* By Pierre Dejours. Pp. xvi+253. (North Holland: Amsterdam and Oxford; American Elsevier: New York, 1975.) Dfl. 35; \$14.75.

HAVING an almost exclusively neurophysiological interest in respiratory movements, I undertook the reviewing of *Principles of Respiratory Physiology* with some trepidation, since I knew this would force me to tread very unfamiliar paths through the jungle of work on respiratory gas exchange in different animals. I am glad to say that my fears were unfounded. True Frenchman that he is, Dr Dejours has created a masterful 'Guide Bleu' through this jungle, providing a clear trail for the timorous, yet offering the more adventuresome rich local detail and source references on which to linger.

I particularly like the way in which he deals with the relevant physical principles of diffusional and convective gas flows, the  $O_2$  and  $CO_2$  capacitances of the gaseous and fluid compartments of the respiratory system and their corresponding  $O_2$  and  $CO_2$  conductances. The operation of these principles in the living organism is illustrated throughout the book by appropriate reference to phylogenetic or species specific mechanisms of gas exchange in both water and air breathers representing the most lowly and most complex of animals. The net result of this endeavour is that Dejours has produced an impressive, overall view of gas exchange even for the most complex Metazoa in which he traces the flow of  $O_2$  from the environment to the mitochondrion by way of the alveolar gas, gas-blood barrier, blood, systemic capillary walls, interstitial fluids and cell membrane, together with the flow of  $CO_2$  in the reverse direction.

The clear authority of the author is no better marked than in the closing chapter where he expresses the view point that the principal aim in the

control of respiration is the adequate oxygenation of the tissues in contrast to the more commonly held view that it is the  $CO_2$  pressure and acid-base balance which are primarily regulated. His defence rests on the evidence that only long term changes in the needs of oxygenation lead to significant adaptive morphological, biochemical and physiological changes of the organism.

Highly recommended to all air breathers interested in respiration.

T. A. Sears

## Electronic structure

*Electron Densities in Molecules and Molecular Orbitals.* (Physical Chemistry: Series of Monographs, Volume 35.) By John R. Van Wazer and Ilyas Absar. Pp ix+101. (Academic: New York and London, September 1975.) \$14.50; £7.25.

THIS is an unusual book. Essentially the authors have a computer programme for Hartree Fock electronic wave function calculations for molecules incorporating flexible possibilities for the basis sets and a perspective three-dimensional graphic output expressing the electron density throughout a cross-sectional plane. This they use for total and for one-electron densities for  $LiF$ ,  $H_2O$ ,  $HCP$ ,  $C_2H_6$ ,  $PF_3O$  and some 30 other molecules. The book largely presents these graphics and associate text for undergraduate chemists "whose minds are spatially oriented"; for the more expert there is little except possible illustrations for a lecture.

A greater attention to detail is necessary, such as clear bold labelling of the molecule for each figure, clear indications of the nuclear positions, clearer depiction of nodal lines, omission of slapdash jargon—such as reference to the  $C_3$  axis of  $PHF_2O$ —and careful spatial orientation of all figures relating to the same molecule such as 3.1 and 3.2 on page 29. Emphasis on the shape of the electron density in a single cross section downgrades the importance of kinetic energy, ignores spin effects, ignores time correlation features associated with configuration interaction and leaves the weaker student with a dominant picture of molecular orbitals as sharp peaks on each nucleus (where  $\psi^2$  remains large for s orbitals) surrounded by a trough (associated with the relatively unimportant spherical nodal surface in 2 s orbitals) and a few small bumps associated with bonds.

Others may agree that there are better ways of introducing simple electronic structure to undergraduates.

D. H. Whiffen



# obituary

**Professor Thomas A. Bennet-Clark**, CBE, FRS died peacefully after a long illness on November 24, 1975. He was born in Edinburgh in 1903 and educated at Marlborough College and Trinity College, Cambridge, where, under the inspiration of F. F. Blackman, he switched from Chemistry to Botany, and won the Frank Smart prize (1923). He started research under Blackman but after a year moved to Trinity College, Dublin, to become research assistant to H. H. Dixon. From 1930 to his retirement in 1967, he held University posts in Manchester, Nottingham, Kings College, London and East Anglia.

Blackman's influence on Bennet-Clark can be seen in his early contribution to plant metabolism, but he went beyond the essentially analytical work of Blackman. Whereas Blackman's analysis of the Pasteur effect in apples made no reference to foreign research, Bennet-Clark was quick to recognise the deep significance of Meyerhof's work on muscles. Bennet-Clark's work on metabolism was a landmark and initiated a series of investigations leading from those of Meirion Thomas on CO<sub>2</sub> fixation to the present recognition of the underlying biochemical unity in the organic acid metabolism of succulents and C<sub>4</sub> plants.

The six years spent with Dixon were particularly happy years. The work at Dublin on cell membranes and permeability led to a study of osmotic relations. This work raised the possibility of the active uptake of water, which excited much interest and inspired a great deal of experimentation in plant biophysics.

Bennet-Clark adapted filter paper chromatography for the assay of activators and inhibitors of plant growth. This work initiated an important series of investigations on plant hormones particularly involving the work of Wain and Wareing. His work on diageotropism (in collaboration with Sir Nigel Ball) did not lead to major developments in plant physiology. The experiments do, however, reveal great ingenuity and originality, and should be read by all young plant physiologists.

Bennet-Clark's contribution to Plant Physiology was highly original but its quantity was not large. Partly this was due to his high regard for quality, partly due to limited facilities, but most of all due to his unselfishness. He was full of ideas, his quick mind produced lateral thoughts at an alarming rate, few, however, were tested experimentally because he would not use his great influence in the ARC and in other quarters to fund his personal researches. This was undoubtedly a

mistake, but given Bennet-Clark's character, it was inevitable.

He was a gentle, kind man with a wry sense of humour and a charming eccentricity which endeared him to the hearts of his students and colleagues. He always found time to help those in difficulty and his advice on both scientific and personal matters was appreciated by many. He was an outstanding teacher and lecturer. He liked to pretend that he knew little taxonomy, but his encyclopaedic mind contained a stock of information on taxonomy and many other topics which were subject to instant recall and association in novel ways.

Bennet-Clark contributed greatly to the success of the Society for Experimental Biology and the Journal of Experimental Botany, of which he was the first editor. His scientific achievements were recognised by his election to the Royal Society in 1950 and his work for the Agricultural Research Council was recognised by the award of a C.B.E. The association of the Food Research Institute and the John Innes Institute with the University of East Anglia is a monument to Bennet-Clark. But his colleagues will remember above all his great personal qualities. We loved him and mourn his passing.

D. Davies

## announcements

### International meetings

March 29–April 2, **Atomic and molecular physics conference**, Belfast (The meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX, UK).

March 31–April 2, **Intermediate moisture food**, Weybridge, Surrey (The Secretary, National College of Food Technology, St George's Avenue, Weybridge, Surrey KT13 0DE, UK).

### Reports and publications

#### Other countries

The Politics and Responsibility of the North American Breadbasket By Lester R. Brown. (Worldwatch Paper No. 2.) Pp. 43 (Washington, DC: Worldwatch Institute, 1776 Massachusetts Avenue, NW, 1975)

Mass Emergencies, Vol. 1, No. 1, October 1975. Edited by J. Nehnevajsa and E. Quarantelli (An International Journal of Theory, Planning and Prac-

tice) Pp. 1–86. Published quarterly Annual subscription Dfl. 86, \$36.75 (Amsterdam Elsevier Scientific Publishing Company, 1975)

History of Antarctic Exploration and Scientific Investigation Plates and Bibliography Compiled by the American Geographical Society (Antarctic Map Folio Series. Folio 19) Pp. 6+15 plates (New York American Geographical Society, 1975.) \$15 plus \$1 postage and handling.

United States Department of the Interior: Geological Survey Professional Paper 763 Stratigraphy of the Inyan Kara Group and Localisation of Uranium Deposits, Southern Black Hills, South Dakota and Wyoming By Garland B. Gott, Don E. Wolcott and C. Gilbert Bowles Pp. iv+57+4 plates (Washington, DC: Government Printing Office, 1974) \$5.10

United States Department of Agriculture. Index-Catalogue of Medical and Veterinary Supplement 20, Part 1, Authors—A to Z By Judith H. Shaw, et al Pp. xvi+558. (Washington, DC: Government Printing Office, 1975.) \$6.15.

United States Department of the Interior: Geological Survey Professional Paper 456–I Geology of the Arabian Peninsula, Jordan By Friedrich Bender. Pp. vi+36+3 plates. Professional Paper 835: Quaternary Geology of Alaska By Troy L. Péwé. Pp. v+145+plate 1. Professional Paper 876 Cauldron Subsidence of Oligocene Age at Mount Lewis, Northern Shoshone Range, Nevada. By Chester T. Wrucke and Miles L. Silberman Pp. ii+20 (Washington, DC: Government Printing Office, 1975.)

Mauritius Sugar Industry Research Institute Annual Report for 1974. Pp. 76+15 statistical tables. (Rédut: Mauritius Sugar Industry Research Institute, 1975)

United States Department of the Interior: Geo-

logical Survey Professional Paper 918: Lithium in Unconsolidated Sediments and Plants of the Basin and Range Province, Southern California and Nevada. By Helen L. Cannon, Thelma F. Harms and J. C. Hamilton Pp. iii+23. (Washington, DC: Government Printing Office, 1975)

United States Department of the Interior: Geological Survey Bulletin 1384: Aeromagnetic and Limited Gravity Studies and Generalized Geology of the Bodie Hills Region, Nevada and California. By F. J. Kellinhampl, W. E. Davis, M. L. Silberman, C. W. Chesterman, R. H. Chapman and C. H. Gray, Jr Pp. iv+38 plate 1. Bulletin 1385-C: Mineral Resources of the San Pedro Parks Wilderness and Vicinity, Rio Arriba and Sandoval Counties, New Mexico By Elmer S. Santor, Robert B. Hall and Robert C. Weisner Pp. vi+29+plate 1. Bulletin 1391-C: Mineral Resources of the Clear Creek-Upper Big Deer Creek Study Area, Contiguous to the Idaho Primitive Area, Lemhi County, Idaho By Fred W. Cater, Darrell M. Pinckney and Ronald B. Stotelmeyer Pp. vi+41. Bulletin 1394-E: The Auld Lang Syne Group of Late Tertiary and Jurassic(?) Age, North-Central Nevada. By D. B. Burke and N. J. Silberling Pp. iii+14. 35 cents. Professional Paper 851: Soil Slips, Debris Flows, and Rainstorms in the Santa Monica Mountains and Vicinity, Southern California. By Russell H. Campbell Pp. iv+51. Professional Paper 871: Lithology and Origin of Middle Ordovician Calcareous Mudmound at Meiklejohn Peak, Southern Nevada. By Reuben James Ross, Jr., Valdar Jaanusson and Irving Friedman Pp. iv+48. Professional Paper 886: Post-Carboniferous Stratigraphy, Northeastern Alaska. By R. L. Dettmerman, H. N. Reiser, W. P. Brosge and J. R. Dutro, Jr Pp. iii+46. (Washington, DC: Government Printing Office, 1975)

**nature***January 29, 1976*

## Have you ever thought of going into industry?

DOES Britain do enough to ensure that the merits of industry as a career are presented to young people?

In the last year the feeble productivity performance of British industry has been brought to public attention with depressing frequency. The figures speak for themselves: in five years the productivity index has risen by only 13%, the index of actual production by less than 2%. As we have noted before, the academic world has been weighing in recently with its own remedies, generally aimed at improving the rather bad university/industry links at the postgraduate end of the spectrum. Raising the productive capacity of industry has also been the recent theme of Mr Fred Mulley, Secretary of State for Education and Science, who has been urging students to think more of industrial careers and less of academe or the civil service.

There are, of course, two fairly distinct routes by which graduates get into industry. One is by making a definite choice around the age of seventeen to pursue engineering at university. The other is by pursuing some other course at university which does not exclude the possibility of working in industry. But it is by no means obvious that the student of seventeen will have enough information available to him or herself to make a reasoned judgement on whether to go into engineering.

Ask any schoolteacher what causes a child to opt for engineering and the most likely answer is that family influences play a major part. Parents or uncles who are themselves engineers seem to be the examples which lead many young people into the profession themselves. In this respect, engineering runs parallel to farming and medicine. The next most common reason for going into engineering is the conspicuous presence of a potential employer, probably in the local town—though three years later the graduate may well have a different opinion about returning home, of course.

Both of these reasons are rather good ones with a high content of realism in them, but what of the student without engineering in the family and with no particular employment in mind? The claims of engineering are, in general, rather poorly represented to him or her. Quite rightly there is a strong insistence in schools on the pur-

suit of mathematics, physics and chemistry, so few engineers are to be found amongst schoolteachers. Small wonder, then, that many pupils will have never seriously considered engineering as an option to be set against a 'science' course at university.

Obviously those who do choose to do science have a chance to enter industry in a variety of ways upon graduating. But their period at university is likely to be one in which they will hear that pay in industry is not as good as it could be, because industry does not prize its graduates. They will also hear that the prospects of a science-trained employee getting to the boardroom are decidedly inferior to those of, say, an accountant. And even if these statements are not universally true, few in the university science environment are in a position to, nor sometimes wish to, speak in defence of industry.

There are many ways in which the recruitment of scientists for industry and the recruitment of students into engineering deserve attention, but the greatest advance surely awaits the removal of the barriers which in most universities separate engineering students from science students. In many cases hardly a course is taken in common even though the subject matter, particularly in mathematics and physics, runs broadly parallel. If a physics department wishes to teach electronics it will call on a physicist to do so; if an electrical engineering department needs a course in solid state physics it will be taught by an electrical engineer. These tendencies are greatest in the older established universities. The result is often inferior teaching, and, worse, the erection of disciplinary barriers by students themselves.

In recent months there has been some fairly radical talk about the need for postgraduate training modelled on the (best) American institutions. These same institutions are offering undergraduates a diversity which is unmatched in Britain and which allows the student to delay the difficult specialisation decision as long as possible. Critics claim that this leads to academically lightweight graduates: it does on the other hand lead to a greater maturity in making decisions about careers. And who can say that in the United States industry has not profited from the flow of excellent graduates? □



**Paul Richards**, coordinator of research for the International African Institute's Environmental Review Unit, examines new approaches to environmental research and explains

## What environmental crisis means in Africa

**E**NVIRONMENTAL crisis has become a major neurosis of the seventies, and monitoring and resource management two of its most commonly advocated therapies. But these therapies mean different things in different parts of the world. For richer industrial countries the focus is on the conservation of fossil fuel resources and on pollution problems. But in poorer regions securing the year's food supply from an increasingly overstretched agricultural system frequently overwhelms all other considerations. Atmospheric pollution and petrol rationing are minor irritations in a context of underdevelopment so severe that up to 50% of all live-born children die before they reach the age of five. For the poorest countries, therefore, dead fish and factory-poisoned rivers might be considered welcome evidence of a turn for the better and a small price to pay for escape to prosperity. In contexts such as these, environmental monitoring will tend to focus on nutritional rather than pollution levels, and resource management must, almost of necessity, emphasise quick exploitation rather than longer term conservation.

The distinction between richer and poorer countries in environmental matters goes much deeper than the mere exigencies of current economic planning, however. Environmental research is often location specific, and poorer regions have had less than their fair share of attention in this respect. This in itself is a factor making for continued poverty. Tropical climatology, for example, is a much less well-researched and closely monitored field than its temperate latitude equivalent, with the result that recent drought in Africa has been much less easy to foresee and its consequences much less easy to plan for. But by far and away the most important distinction is that, by taking part in the world trading system, richer nations have been able to diversify and build flexibility into their economies while poorer nations have found themselves beset by a process of structural simplification rendering them less able to withstand environmental shock. Thus the export crop plantation and pesticide spray replace the ecologically subtle polyculture of the subsistence farm with its natural checks and balances. Not only is the new system more vulnerable to pests and diseases, but many plants, skills and habits associated with a sub-



Drought in the Horn of Africa (Photo: Keystone)

sistence way of life disappear irretrievably. Should famine then occur for whatever reason—political or natural—suffering is likely to be much greater.

The subsistence farmer is a natural survivor under conditions of ecological crisis—his answer to the destruction of his crops by grasshopper or locust is to feed on insects for a time. Development pushes in the opposite direction. I have vivid memories of a village meeting on nutritional problems in Nigeria being forced to disband under the bludgeoning of the bulldog amplifier on a van selling patent medicines for an international pharmaceutical concern. When advertising can penetrate—in this way or via radio—to the most isolated rural areas in Africa, then the imported product of modern manufacture enjoys a powerful advantage over local equivalents. Perfectly acceptable local foodstuffs are traded for volumetrically inferior amounts of processed food or totally inappropriate medicines and tonics, and children malnourished on packaged baby foods then become innocent casualties of a world in which the white-coated image of scientific authority on the advertising hoarding is used to undermine confidence in the old ways for the sake of commercial progress.

In Africa the old relations between man and his environment still have much to tell us. The 'alternative technology' movement in industrially advanced societies is struggling to recover some of the self-sufficient technology of an earlier generation.

In Africa the crops, cultivation practices, crafts and medicines that support a largely self-sufficient way of life are still part of a living tradition, but a major research effort is now required to establish and preserve this knowledge and skill in permanent form. If growing ecological consciousness in science can lead to a greater awareness of and interest in endangered plants and animals, then surely tropical farming systems, pharmacopaeias and technologies deserve at least equal attention. The task is one of trying to encourage, co-ordinate, publish and where necessary to initiate research into aspects of man-land relationships and pre-industrial technology endangered and discriminated against by processes of 'modernization' and economic development in Africa, in the hope that viable 'alternative' strategies of environmental resource management can thereby be identified.

There is, in fact, a fair amount of activity in this sphere in Africa at the present time, but it is a difficult field in which to work for a number of institutional and organisational reasons. Colonially imposed boundaries and *lingua franca* do not follow ecological lines. Scientists in neighbouring countries may be working on comparable environmental problems but fail to benefit from each others' progress for two reasons: firstly, the anglophone/francophone (not to mention lusophone) barrier is even more difficult to transcend in Africa than it



is in Europe, due to lack of language teaching facilities at secondary school, hardly made good by perfunctory courses in French, English and so on for scientists at university level; and secondly, it often appears easier for an African scientist to arrange study leave and research facilities in Europe or North America than in a neighbouring African country. Comparative approaches, in consequence, are rare, and good local case studies tend to remain local in their impact. But above all, the major difficulty lies in the nature of the work, straddling the divide between social and natural science—an unfortunate line of demarcation hallowed not by logic but by usage.

Leaving aside the arid social-antisocial, natural-supernatural science arguments, there are genuine differences of style and method between social and natural scientists—social scientists, for example, are part of what they study and their language leans towards the polemical in the hope of influencing their material for good. Such differences have to be overcome before the two groups can work together effectively. And yet ethnobotany, 'folk' medicine and 'appropriate' technology would be unthinkable without collaboration between, say, biochemists, ecologists, agronomists and engineers on the one hand and linguists, ethnographers and geographers on the other.

The Environmental Review Unit of the International African Institute is one body trying to find its way across these various barriers and obstacles, partly through its bilingual publications programme, which is based on a working relationship between the

Training for the Environment Programme in Dakar and the IAI (the journal *African Environment/Environnement Africain* and the *Special Report* series of monographs and collections of essays are joint ventures, for example), and partly through a research programme which emphasises the involvement of African scientists and scholars in cross-national studies of environmental problems from a comparative and interdisciplinary point of view. The IAI's traditional strength lies in the linguistic and social science fields, but this is being complemented by links with institutions in Africa having strong natural science representation.

Three major research projects are in the course of being established. The first involves the study of nutritional concepts and strategies in subsistence societies and how these are changing, for better or for worse, under the impact of agricultural development. The second is in a way linked, being an attempt by a group of archaeologists, ethnobotanists and ethnographers to investigate the origins as well as the present-day significance of yam cultivation in Africa. The third project involves looking at the agronomic and ecological conceptions of peasant farmers to assess the role they might play in community-based programmes of environmental monitoring and resource management in Africa; it is based on collaboration between IAI research workers in Africa and the Monitoring and Assessment Research Centre of Chelsea College, London, with initial funding from SCOPE, and has resulted in a preliminary report of a Nigerian case study.

The funding of such research, which falls between the natural and the social sciences, poses problems of its own. But with initial results exhibiting great promise for further development along the lines outlined, the hope is that those who normally fund research on either the social or the natural science side will stretch their terms of reference to cover this vital area. The financial resources required are relatively modest but, even so, no single institution or individual can assemble and effectively combine the range of skill and experience required. Inter-disciplinary co-operation rather than intellectual rivalry is the essential pre-requisite. World wide studies by the Disaster Research Unit at the University of Bradford indicate that, while the probability of the occurrence of geophysical and climate disturbances has remained constant over the last hundred years, 'disasters' have doubled.

The conclusion is plain—namely that human communities are increasingly vulnerable to natural hazard, for social rather than natural reasons. Players of the environmental game appear to have two strategies open to them—minimising maximum losses or maximising potential gains. The modern world opts for the latter in the interests of growth and profit. But someone has to pay the price for this aggressive competitiveness, and it is the poor who end up more vulnerable to ecological breakdown than ever before. Good environmental research is difficult to do because it requires a direct reversal of precisely those qualities of individual rivalry and competitiveness that lie at the root of our present environmental predicament. □

## US BUDGET

# Escaping the 'New Realism'

Colin Norman explains how science and technology fare

WHEN President Ford unveiled his election year Budget last week, he said it reflected "New Realism" in government policy in the United States. The new realism turned out, however, to be old-style conservatism in the shape of proposals for massive increases in military spending, swingeing cuts in government expenditure in fields such as health and social services, and tax relief for individuals and corporations. Many of those proposals will be unpalatable to the democratic-controlled Congress—which must act on them before they become law—and thus, as a guide to what will actually be spent by government departments and agencies in the 1977 fiscal year (which begins on October 1, 1976), Mr Ford's budget

figures should be treated with considerable caution.

Nevertheless, the budget, which consists of thousands of pages of facts and figures, richly laced with promises and rhetoric, is an important statement of the Administration's political thinking; it sets out in fine detail the programmes for which Mr Ford and his Administration will seek congressional approval in the coming months. As far as science and technology are concerned, the Administration's thinking seems to be surprisingly expansive in view of the frugality displayed elsewhere in the budget.

The extensive axe-wielding which resulted in keeping the total budget request within Mr Ford's target of



Gerald Ford: 'realist'

\$395,000 million for the federal government next year, left most areas of research and development relatively unscathed. In fact, Mr Ford has proposed hefty increases in several areas



of science and technology, though there are notable exceptions in a few areas, such as biomedical research and space science.

Putting the figures in their best possible light, Dr H. Guyford Stever, the President's science adviser and Director of the National Science Foundation (NSF), noted last week that the budget contains a total of \$23,500 million for research and development and a further \$1,200 million for scientific equipment and facilities. If Congress agrees to the figures, and if all the money is spent, that would amount to an increase of some \$2,200 million (11%) over anticipated expenditures this year. With inflation now reckoned to be about 6 or 7%, the budget proposals would actually result in some real growth in federal support for science and technology.

The increases are particularly unexpected because federal expenditures on research and development fall almost entirely in the portion of the budget which is relatively controllable. More than half the total budget is now spent on such items as pensions and medical payouts for the poor and elderly, which cannot easily be cut, and thus Mr Ford had to look for most of his savings in the relatively controllable section. Using a bit of the rhetoric which is customary on such occasions, Dr Stever told reporters last week that the fact that science and technology fared well in the budget suggests that "this Administration has clearly placed a high priority on research and development for the achievement of national goals".

Two of the Administration's goals—beefing up military technology and securing long-term energy independence for the United States—would swallow up the lion's share of the increases. But another, more surprising trend emerges from the budget pro-

posals. Basic research, federal support for which has dwindled since the late 1960s, would get a monetary shot in the arm, increasing by about 11%. The National Science Foundation's research budget alone is set for an increase of about 20%, and anticipated support for research and development in colleges and universities is set for a 9% boost.

Within that overall budget, the Administration has decided to give its backing to a number of large new science projects. There is money in the budget to begin construction of a \$78 million positron-electron colliding beam device at Stanford University, NASA has been given the green light to begin building a solar spacecraft to study the maximum sunspot activity in 1979-80, and several new energy projects have been proposed.

But there are also bleak spots. Congress and the Administration have not yet been able to agree on the size of the budget for the National Institutes of Health (NIH) for this year, let alone next year, and a lengthy tussle seems inevitable. Ford, in short, wants to trim this year's budget for NIH below what it was in 1975, and he has proposed only a modest increase above the 1975 budget for 1977 (*see later*). Congress on the other hand, wants to increase the NIH budget substantially. The outcome of that tussle will profoundly affect the entire picture for support of research and development by the federal government, particularly in the universities which receive nearly half their total funds for federal research from NIH. Furthermore, NASA has had to defer plans for several key science projects, including the large space telescope, and the Environmental Protection Agency's research and development budget has suffered from the Administration's knife.

Another area which is troubling

many government science agencies is that Mr Ford wants to cut the size of the civil service, which means that although some agencies may get larger research budgets, they will have to administer them with fewer people.

How is Congress likely to treat the science budget? Traditionally, the Administration's budget request is picked over piecemeal by a number of appropriations subcommittees, each of which handles the budget for one or two departments or agencies. Consequently, it has been difficult for Congress to keep track of the size of the total budget, and it has not been easy for Congress to set priorities for some departments in relation to others. But this year, for the first time, Congress has a fully operational budget system to combat those deficiencies. A Budget Committee will establish an overall target figure for the entire federal budget (which may or may not be the same as Mr Ford's target figure), and it will set limits for each individual appropriations subcommittee to stick to. Though the Budget Committee is likely to sympathise with Mr Ford's attempt to reduce the size of the federal deficit—the Administration's budget anticipates that the deficit will shrink from \$76,000 million this year to \$43,000 million next—it will probably attempt to do it in a different way. In short, the major budget battles are likely to centre on the size of the defence budget, with Congress seeking to reduce it below Mr Ford's proposed level, and the size of the budget for the Department of Health, Education and Welfare, which Congress will try to increase. Since those two departments fund the bulk of federal research and development, the outcome of those battles will shape the overall picture for science and technology.

The following are the major highlights in the budget for science and

Table 1 Conduct of research and development of major departments and agencies (in \$ million)

Department or agency	Obligations			Outlays		
	1975 actual	1976 estimate	1977 estimate	1975 actual	1976 estimate	1977 estimate
Defence—Military functions	8,987	9,879	11,198	9,189	9,468	10,762
National Aeronautical and Space Administration	3,088	3,473	3,573	3,181	3,402	3,550
Energy Research and Development Administration	2,071	2,812	3,282	1,862	2,423	3,042
Health, Education, and Welfare	2,395	2,369	2,570	2,108	2,366	2,512
National Science Foundation	604	628	726	571	602	647
Agriculture	424	483	507	418	486	510
Transportation	291	340	319	307	338	304
Interior	296	332	316	265	307	310
Environmental Protection Agency	258	305	241	207	324	298
Commerce	222	247	243	220	239	233
Veterans Administration	99	108	106	97	99	100
Nuclear Regulatory Commission	61	97	109	54	88	103
Housing and Urban Development	57	62	70	52	57	67
Justice	44	65	41	44	50	44
All other	126	138	164	124	142	156
<b>Total</b>	<b>19,023</b>	<b>21,338</b>	<b>23,465</b>	<b>18,699</b>	<b>20,391</b>	<b>22,638</b>
<b>Total, conduct of research</b>	<b>6,759</b>	<b>7,150</b>	<b>7,782</b>	<b>6,355</b>	<b>7,192</b>	<b>7,709</b>
<b>Total, conduct of development</b>	<b>12,264</b>	<b>14,188</b>	<b>15,683</b>	<b>12,344</b>	<b>13,199</b>	<b>14,929</b>



technology, and the likely congressional arguments.

### National defence

Claiming that "we dare not do less", Mr Ford has proposed a record-breaking budget for the Department of Defence (DOD), amounting to \$114,000 million, of which some \$101,000 million would actually be spent in the 1977 fiscal year. Consequently, military research and development in DOD is set for a whopping budget increase, from about \$9,900 million to \$11,200 million. In addition, Mr Ford has proposed that the Energy Research and Development Administration (ERDA) should spend another \$775 million on nuclear weapons research. Thus, according to Ford's proposals, military research and development would carry off more than half the total science and technology budget, and its share would actually increase.

Most of that money would be spent on weapons development, with ballistic missile projects receiving highest priority. Defence-related basic research is projected to rise from \$330 million to \$383 million. DOD is also under an edict to reduce its civilian staff by some 26,000 which could have repercussions in defence laboratories, and it could also result in more defence work being performed in colleges and universities.

Congress is unlikely to approve an overall defence budget of that size, however, and a major political battle over several weapons programmes should be anticipated.

### Energy research and development

The second area to be favoured with large proposed increases is support of energy research and development programmes, most of which are now clumped in the Energy Research and Development Administration. Mr Ford's budget includes some \$3,200 million for direct energy research and development, together with closely related environmental and basic research. That would amount to an increase of nearly 40%. Congress is not likely to quibble about the fact that the budget for such efforts is increasing by leaps and bounds, but it is likely to question the way in which the money is to be divided between nuclear and non-nuclear technologies.

The Ford budget proposes a sharp increase in spending on fission reactor development and related studies on the fuel cycle, from \$852 million this year to \$1,215 million next. A good deal of that increase would be swallowed up by the Liquid Metal Fast Breeder Reactor (LMFBR) programme, which alone would receive \$655 million next year. That heavy emphasis on the

LMFBR is consistent with a recent policy statement by ERDA administrator Dr Robert C. Seamans Jr, who said that after studying the environmental, economic and technological questions surrounding the breeder programme, he has decided that ERDA should proceed rapidly with LMFBR development. The goal is now to have the first commercial LMFBR in operation by 1993.

The other noteworthy increase in the budget for energy research and development is in the thermonuclear programme. Mr Ford has proposed that the fusion programme should receive a total of \$392 million next year, a massive boost from this year's budget of \$250 million. A large part of the increase is earmarked for the Tokamak fusion test reactor, a major facility under construction at Princeton University which should be in operation by about 1982. The laser fusion programme is also set for a substantial increase, from \$83.6 million to \$101 million.

Large percentage increases have also been proposed for non-nuclear technologies, such as solar energy, development of oil shale, geothermal power, and conversion of coal to liquid and gaseous fuels. But they would continue to receive much less than half the total energy research and development budget.

Congress is likely to question the increased commitment to nuclear energy which the budget entails, but nuclear opponents in Congress probably lack the votes to reduce the nuclear budget substantially. A good deal of noisy skirmishing should be anticipated, however.

### Biomedical research

Once again, the big loser in the federal research and development budget seems to be biomedical research. The situation is, however, greatly complicated by the fact that Congress and the Ford Administration have been unable to agree on the size of the budget for the Department of Health, Education and Welfare (HEW)—of which NIH is a part—for this year, and until they do, the outlook for biomedical research support is very uncertain.

Late last year, Congress passed a bill for HEW which contains very large increases in Mr Ford's original budget request for NIH in the 1976 fiscal year, which is already well under way. Mr Ford vetoed the measure, however, because he claimed that it would fuel inflation and instead, he has now proposed that NIH should receive slightly less money this year than it received last. And the budget he has proposed for 1977 would not even restore NIH to the 1975 level if inflation is taken into account. The various budget pro-



*Dr H. Guyford Stever, NSF Director*

posals for NIH are shown in Table 2.

Congress will try this week to override Mr Ford's veto of the HEW bill. If it is successful, then NIH would receive a hefty budget increase this year. Otherwise, if NIH is funded at the level proposed by Mr Ford, there would be insufficient money available to fund any new projects until next October. In other words, support for new biomedical research would grind completely to a halt. Moreover, NIH budget officials have calculated that Mr Ford's proposed budget for next year would allow them to fund only 37% of the grant applications approved by peer review groups. The outcome of Congress's attempt to override the veto is difficult to predict, but the prevailing opinion on Capitol Hill seems to be that it will be successful.

Nevertheless, Mr Ford's budget proposal for 1977 shows a very significant new trend. For the first time in five years, the Administration has suggested that funds for cancer research should be held in check while support for other areas of biomedical research should be allowed to grow. That suggestion follows months of debate about relative priorities in biomedical research, centred on the fact that cancer research funds have grown by 280% between 1973 and 1975, while NIH's budget for other research has increased by only 20% in the same period.

It is questionable whether Congress will go along with such a redistribution of funds for biomedical research, but at least the proposal would sharpen discussions of research priorities.

Another problem in NIH's proposed budget is that it contains no provision for increases in NIH staff positions. According to Dr Donald Fredrickson, Director of NIH, that restriction could be especially burdensome because NIH is starting up an entirely new institute devoted to study of the ageing process, and it will be forced to do it without any new people.



Congress and the Administration should reach some agreement—or at least a stalemate—on NIH's 1976 budget in the next few weeks. The tussle will then begin over the size of the budget for next year, all of which makes for extremely difficult planning not only at NIH but also in the universities, which now receive about \$1,300 million a year in grants and contracts from NIH.

## The National Science Foundation

The bulk of federal funds for basic research come from the National Science Foundation (NSF). Its budget is therefore closely watched in the scientific community as a barometer of federal support for basic research. About 87% of NSF's money is spent on grants to scientists in colleges and universities.

The budget proposed for NSF would result in a total increase of about 11%, reaching \$812 million. Within that total, NSF's basic research programmes would grow by nearly 20%, while its applied research and education support would either decrease or remain constant. According to Dr Stever, the "specific aim" of the proposed increases "is to counteract the gradual decrease in federal support for basic research which has declined by about 23% in terms of constant dollars since 1968".

The proposed increases in research support would be applied more or less evenly throughout the sciences. No new large projects are planned, but the budget contains sufficient money to continue construction on schedule of the very large array telescope system which is being built in New Mexico.

Usually, NSF's budget attracts little attention in Congress, and aside from minor tinkering by the appropriations committees, the Foundation receives close to the amount of money requested. But last year NSF came in for very heavy criticism on Capitol Hill from Congressmen and Senators who believed that it was either supporting trivial research or that it was developing morally unacceptable school science courses. Consequently, Congress cut NSF's budget for this year, and arranged that basic research bore the brunt of the reductions. Close scrutiny of NSF's programmes can be expected again this year, and Stever warned last week that if the decline in basic research support is to be halted, "Congress must rally round this budget".

## Space science

Once again, the National Aeronautics and Space Administration (NASA) has been cut back and space science has been squeezed especially hard. Dr James Fletcher, the Administrator of NASA, said last week that the proposals for NASA mean that 1977 "will be another year of tight constraints, minimal budgets and limited new activities".

The chief problem is that NASA's total budget is being held approximately constant, while expenditures on the space shuttle are increasing rapidly. The net result is that the shuttle is soaking up a growing share of the Agency's budget, leaving little to spare for space science. Next year, for example, out of a total of some \$3,676 million proposed for NASA, the space shuttle will account for \$1,288 million.

The effects of the squeeze will be particularly severe in the budget for

the Office of Space Science, which is set to shrink from \$417 to \$379 million. Only one major new start has been proposed for next year—a so-called solar maximum mission, a spacecraft which will study solar flares and other phenomena during the period of peak solar activity in 1979–80. The chief casualty is the Large Space Telescope (LST), an optical telescope which NASA hopes to fly on an early shuttle mission in the early 1980s. The LST has been accorded top priority by the Space Science Board of the National Academy of Sciences, and NASA was hoping to begin developing it this year. A start has now been deferred, however, until at least 1978.

Another high priority space science mission which will have to wait for at least another year is the proposed mission to send an orbiter and probe to Jupiter in 1981. Again, there is no money in the budget for that mission, but NASA officials are hoping to make a start in 1978. But one planned venture has been scrapped entirely—the proposal to send a Mariner spacecraft to Jupiter and then on to Uranus. Because there is no money in NASA's budget to start work on that mission next year, the opportunity to make use of a rare alignment of the outer planets to send a spacecraft past Jupiter and on to Uranus will be lost. NASA officials are hoping, however, to reprogramme an already-approved spacecraft due to be launched next year, so that it will fly close to Uranus in 1985 after it swings past Saturn.

In past years, Congress has not made many substantial changes to NASA's space science budget, and there is no reason to expect any difference this year. □

Table 2 National Institutes of Health (in \$ thousand)

Institute or Division	1975 Actual	1976 Revised President's Budget	Vetoed 1976 bill*	1977 President's Budget	1977 change over 1976 President's Budget
Cancer	691,666	\$687,394	743,564	\$687,670	+ 276
Heart	324,630	304,702	349,059	342,855	+ 38,153
Dental	50,033	48,592	45,794	52,207	+ 3,615
Arthritis	173,514	161,843	175,172	180,837	+ 18,994
Neurology	142,498	135,139	136,546	146,532	+ 11,393
Allergy	119,452	119,136	118,918	135,615	+ 16,479
General Medical Sciences	187,400	167,538	146,461	193,435	+ 25,897
Child Health	142,435	122,174	126,889	129,883	+ 7,709
Ageing	—	16,071	17,526	26,220	+ 10,149
Eye	44,133	44,435	45,565	46,950	+ 2,515
Environmental Health	35,171	34,023	35,915	46,141	+ 12,118
Research Resources	127,200	83,376	129,931	92,342	+ 8,966
Fogarty Center	5,859	5,404	5,705	7,492	+ 2,088
Library	28,850	29,277	29,065	35,234	+ 5,957
Office of the Director	17,326	18,370	17,896	16,234	– 2,136
<b>Total, Biomedical Research</b>	<b>2,089,897</b>	<b>1,977,474</b>	<b>2,124,006</b>	<b>2,139,647</b>	<b>+ 162,173</b>
Buildings and Facilities	3,000	3,000	54,000	25,400	+ 22,400
<b>Total, NIH</b>	<b>2,092,897</b>	<b>1,980,474</b>	<b>2,178,006</b>	<b>2,165,047</b>	<b>+ 184,573</b>

\* Does not include money for training grants, which is included in other columns. Congress has voted a separate budget of \$124 millions for biomedical training in 1976, which would bring the total for biomedical research in the vetoed bill to \$2,300 million.

## UK REPROCESSING CONTROVERSY

# Suffering Fuels gladly

It is all down to a Cabinet decision. The media have had their field day, the antinuclear lobbies have voiced their objections and the Select Committee on Science and Technology has declined to investigate the issue. If British Nuclear Fuels Limited (BNFL) gets the green light it wants in the near future the few remaining wrinkles will be ironed out of the deal with the Japanese Enrichment and Reprocessing Group, and irradiated nuclear fuel from Japan will be all but on its way to Britain for reprocessing.

Although spent Japanese fuel is already reprocessed in Britain, few people doubt that the Secretary of State for Energy, Mr Anthony Wedgwood Benn, aimed to give this deal, involving the reprocessing of 4,000 tonnes of fuel over the decade from 1979, a wide public airing. The recent, unattributed "leak" to the popular UK press put the issue before a wider audience than was already following its progress in the up-market newspapers. Speculation over Mr Benn's own attitude added zest to the coverage. And when BNFL followed a public debate in Barrow with another in London this month the presence of Mr Benn ensured a glare of publicity that opponents to the deal could not afford to miss.

But the odds have been stacked against them from the start. Mr Benn has promised that the government will weigh the issues raised by the debate in making its final decision. But the economic benefits which the deal offers, the essential role of reprocessing in Britain's future nuclear programme, and the excellent safety record of the British nuclear industry, all point to a decision in favour of BNFL.

Nor is there any chance that this particular deal will be affected by suggestions that nuclear reprocessing be conducted on a non-commercial basis through an international arrangement. The Pugwash symposium to outline plans towards that end is still some months off; even then it may take several years before proposals percolate through to effective executive levels. The suggestion is anyway irrelevant to concerns about the wisdom of importing foreign radioactive material to Britain. The BNFL reprocessing plant at Windscale in Cumbria, where the Japanese will send their fuel, with its established facilities and 25 years of operational experience, makes it a prime choice for internationally supervised reprocessing.

Most critics believe that reprocessing can actually be avoided altogether.

Some have suggested that benign energy resources be developed to replace nuclear energy, a view more widely regarded as unrealistic, in the short-term at least. Those who accept the need for a continuing reliance on nuclear power, the Pugwash group among them, suggest that a switch to the thorium cycle would provide a viable alternative to reprocessing. That, however, ignores Britain's commitment to the fast breeder reactor. The first FBR will be on-stream by the turn of the century and reprocessing will be an essential part of their nuclear fuel cycle. Moreover, only the most optimistic interpretations of known uranium reserves suggest that there are sufficient natural resources to fuel second generation reactors until FBRs come into their own. The shortfall, it is argued, must inevitably come from reprocessing.

Another factor in Britain's decision, inevitably, will be finance. Throughout the final rounds of its publicity campaign, BNFL has made great play of the deals' commercial advantages. Apart from attracting other foreign customers, mostly in western Europe, the state-owned BNFL could add an estimated £600 millions to Britain's import earnings on completion of the deal. Though the finer details are still under negotiation it is certain that a substantial proportion of that amount—probably between £100–200 millions—will come as a downpayment on the signing of the contract. Payment of the remainder will be spread over the ten-year period covered by the deal. With each consignment sent to Windscale the Japanese will pay an agreed proportion of each batch's reprocessing cost, the total to be made up when the reprocessed material is returned to Japan.

There is no danger that the Japanese fuel will remain in Britain indefinitely. Should BNFL fail to develop the vitrification technique which will allow them to glassify the reprocessed material for relatively safe transport, they will withdraw from the deal and the Japanese will repossess the fuel untreated. The Japanese Enrichment and Processing Group are apparently satisfied with that condition. BNFL confidently expect that their recent step-up of vitrification research will ensure the viability of the process by the mid-1980s.

The reprocessed material will contain a small but significant amount of plutonium, a key element of nuclear weaponry. But suggestions that in completing the deal Britain will thereby

contravene the Non-Proliferation Treaty are ill-founded. The present signatories unanimously recognise that the present agreement is not intended to ban nuclear trade with outsiders; moreover, Japan is in a position to ratify the treaty at any time. In addition, under a system administered jointly by the International Atomic Energy Agency and Euratom, a series of detailed arrangements, to which the parties involved must accede, provide safeguards for nuclear trade agreements. The safeguards are specifically designed to prevent the clandestine diversion of material to military uses, and allow the IAEA to conduct checks at any time. It was by entering into a similar agreement that West Germany was recently able to sell a complete nuclear fuel cycle to Brazil. Similar provisions will apply to the reprocessing deal between Japan and Britain.

There is another area of uncertainty relating to the reprocessing itself. Critics are concerned because the Japanese, who have now adopted the American light water reactor, will send oxide fuel, and not magnox fuel, to Britain. Most of the foreign fuel so far reprocessed at Windscale, coming in the main from Latina in Italy and Tokai Mura in Japan, has been magnox from first generation reactors. The reprocessing of oxide fuel poses greater problems because of the higher activity of the waste. BNFL has gained little experience in oxide reprocessing and what they have is not entirely encouraging. The first oxide reprocessing plant is yet to be built at Windscale, though up until 1973 BNFL reprocessed minor amounts of foreign oxide fuels using a head-end adaptation on the existing magnox plant. The operation was discontinued following a minor incident in which a small cloud of radioactive dust escaped during servicing, contaminating workers. Nonetheless, BNFL aim to have two plants operational by the mid-1980s, handling 1,000 tonnes of material annually. Most of it will come from abroad, according to the company's projections, though all but a small percentage will return to the country of origin.

Trades union officials in Cumbria are eager to see the deal go through. For them at least the issues are clear cut. The deal is likely to create an estimated 2,000 jobs in the area—at the sizeable projected cost of some \$900 million. This the Cabinet may also consider relevant in its deliberations: BNFL itself has deployed the jobs argument in support of its view. But this issue aside, few people doubt the final outcome.

**Allan Piper**



# correspondence

## Journal guidelines

SIR,—In your issue of November 27, you ask for comments on "Journal guidelines", a discussion which you began in your issue of November 6. You may be interested in publishing the experience with our new journal, *Intervirology*, which is owned and operated by the Virology Section of the International Society for Microbiological Societies.

The journal has 12 sections, each with its own editor and specialised board of reviewers, who are listed in each issue. For the first five volumes, each author selected the appropriate section of the journal and submitted one copy of the paper to the Section Editor and a second copy to a board reviewer of his choice. In this manner, the author was confident that his paper was being judged by persons whom he regarded as authorities in the area of the work being presented. The board member sent his review to the Section Editor, who also reviewed the paper and then made the decision to accept, modify, reject, or seek additional, anonymous review. The final judgement as to publication was made by the Section Editor.

At the Third International Congress for Virology held in Madrid in September, it was decided to modify this procedure. To provide a consistent balance between known and anonymous reviewers, authors are now required to send one copy of their paper to an editorial board member. Essentially, the reviewer acts as the friend in court whom the author respects as an authority in the field. The author also sends two copies to the Section Editor, who reads one copy as before, but who now regularly sends out a copy for anonymous review. As before, reviews are sent to the Section Editor, who continues to make the final judgement on publication.

The editors hope that the current procedure will satisfy authors that they are fairly represented in the decision-making process and at the same time will allow for non-personal critical evaluation before a paper is accepted or rejected.

Yours faithfully,  
JOSEPH L. MELNICK  
(Editor-in-Chief)

Department of Virology,  
Baylor College of Medicine,  
Texas 77025

SIR,—It is extraordinarily difficult to prove the functional value of a complex and subtle social convention such as the preservation of the anonymity of editorial referees. What, for example, is the nature of the 'editorial experience' that Dr C. N. Davies claims (December 18) to confirm his opinion that this long-established custom can be abandoned? His experiment with the *Journal of Aerosol Science* is to be commended; but we shall need to know more about the results before accepting his personal opinion that they are convincing.

Until such empirical evidence is available, I cling rather to the conventional wisdom that anonymity protects the referee from the temptation of softening his remarks to avoid causing personal offence. Most scientists are intellectually conscientious, and will exercise their critical faculties fairly on any paper within their competence; they are seldom (if ever, in my experience) malicious or dishonest. But they know that the delicate competitive/cooperative relationship between colleagues in the same 'Invisible College' cannot stand the stresses that would arise if Dr A had to express, in his own name, in writing, his opinion of the earnest efforts of his rival/friend Dr B. Inevitably, punches would be pulled, and fundamental critical issues would be ducked.

Anonymity is better for all concerned: for the referee, who does not have to mix emotional factors into his intellectual judgements; for the editor, who gets a more honest opinion to guide his decisions; for the reader, who gets more reliable and better expressed papers that have been subjected to a higher standard of criticism; and, strangely enough, for the author who, when his mistakes are pointed out, can vent his chagrin harmlessly in the direction of an impersonal critic without falling into the mortal sin of acquiring a supposed enemy.

The cry against 'authoritarianism' and 'elitism' is, of course, populist in that it appeals to the immediate interest of each individual against the social constraints imposed by the community. But the 'anonymous referee' is only oneself, on the other side of the hill, wearing another uniform. Merton and Zuckermann (*Minerva*, 9, 66; 1971) have found no abuse of the referee system by senior scientists—or whoever the bogies are supposed to be. My own

experience is that the one-way mirror of anonymity facilitates a psychological role reversal, from author to referee, which makes better, more humble, more sceptical scientists of us all, and curbs the vanity and pride of those who claim 'authority'.

This, somewhat schematically and cryptically, is the basis of my assertion that it is a populist folly to disclose the names of referees to authors.

Yours faithfully,

JOHN ZIMAN

University of Bristol, UK

## Unit proposal

SIR,—We would like to propose a unit for electrophoretic mobility ( $u$ ) to replace the current practice of specifying it, for example, as follows:  $u = -2.45 \times 10^{-5} \text{ cm s}^{-1}/\text{V cm}^{-1}$  (+ for cations, — for anions).

In honour of Arne Tiselius, who has done more than anyone else to advance electrophoretic methodology, we propose the adoption of the Tiselius Unit:  $1 \text{ TU} = 10^{-5} \text{ cm s}^{-1}/\text{V cm}^{-1}$  as the unit of the electrophoretic mobility. The above example for electrophoretic mobility will thus be written:  $u = -2.45 \text{ TU}$  in the proposed notation.

This recommendation relies on analogy to the adopted Svedberg Unit  $S$  for sedimentation coefficients ( $s$ ):  $1 S = 10^{-13} \text{ s}$ , where the sedimentation coefficient

$$s = \frac{dr/dt}{\omega^2 r}$$

measures the sedimentation velocity of a particle in a unit centrifugal field, by analogy with the electrophoretic mobility  $u$  measuring the electrophoretic velocity in a unit electrical field.

We will now adopt the Tiselius Unit in our publications and urge others to do so, not only to avoid the wastefulness of the present notation, but also to commemorate the scientist who initiated the far-reaching development of analytical and preparative electrophoretic methodology.

Yours faithfully,

NICHOLAS CATSIMPOULAS

Massachusetts Institute of Technology  
STELLAN HJERTEN

University of Uppsala

ALEXANDER KOLIN

University of California,  
Los Angeles

JERKER PORATH

University of Uppsala,  
Sweden

# news and views

## Self regulation of membrane receptors

from Martin Raff

CELLS communicate with each other largely by means of such chemical signals as hormones, neurotransmitters, and growth factors, most of which bind to specific cell surface receptors. The study of these receptors has been revolutionised in recent years by the development of radiolabelled ligands which bind specifically to particular receptors. One of the rewards of this new technology has been the discovery that the concentration of a ligand can regulate the concentration and/or binding properties of its own receptors on the surface of target cells (see Tata, *Nature*, **257**, 739; 1975). There seems little doubt that this is an important homeostatic regulatory mechanism in cell communication, which, until recently, has been largely overlooked.

The first indication that the binding of a ligand could induce the disappearance of a cell surface macromolecule was provided by Beale (*Int. Rev. Cytol.*, **6**, 1; 1957), who showed that antibodies could specifically remove surface antigens from *Paramecia*, probably by causing them to be shed. Several years later, Boyse and Old and their colleagues observed that antibodies against the Thymus-Leukaemia (TL) antigens caused their disappearance from the surface of thymus and leukaemia cells, both *in vivo* (Boyse *et al.*, *J. natn. Cancer Inst.*, **31**, 987; 1963) and *in vitro* (Old *et al.*, *J. exp. Med.*, **127**, 523, 1968). This phenomenon, referred to as 'antigenic modulation', allowed the leukaemia cells to escape from the host's immune response. A similar phenomenon was later described for H-2 antigens and immunoglobulin (Ig) molecules on mouse lymphocytes (Takahashi, *Transplant. Proc.*, **3**, 1217; 1971). By the use of fluorescein (Taylor *et al.*, *Nature new Biol.*, **233**, 225; 1971) and ferritin-coupled (de Petris and Raff, *Eur. J. Immun.*, **2**, 523; 1972) anti-Ig antibodies, it was subsequently demonstrated that modulation of surface Ig molecules (which serve as antigen-specific receptors) on B lymphocytes was due to pinocytosis of the antibody-

antigen complexes; the same mechanism probably accounts for TL modulation (Yu and Cohen, *J. Immun.*, **112**, 1296; 1974). Antigenic modulation is dependent on time, temperature, and antibody concentration; it occurs progressively over several hours and is prevented by various metabolic inhibitors, but not inhibitors of protein synthesis. If modulated cells are cultured in the absence of antibody, the surface antigens return in 6-24 h; and this requires new protein synthesis (Lor *et al.*, *Eur. J. Immun.*, **2**, 203; 1972). Monovalent Fab fragments of antibodies modulate TL antigens (Lamm *et al.*, *J. Immun.*, **101**, 99; 1968) and induce pinocytosis of surface Ig (de Petris and Raff, *Nature new Biol.*, **241**, 257; 1973), which suggests that modulation does not always require cross linking by the ligand, and this distinguishes it from ligand-induced patching and capping of surface macromolecules, for which cross linking is obligatory (de Petris and Raff, *Nature new Biol.*, *op. cit.*).

Although antigenic modulation has provided insight into some properties of cell membranes, its biological significance is still unclear. Recently, remarkably similar phenomena have been observed when some hormones bind to their membrane receptors; the functional implications here are much clearer, the molecular mechanisms less so. Roth and his colleagues have shown both for mice and for men that when serum insulin levels are chronically elevated (for example, in genetic and induced obesity) there is a striking decrease in specific <sup>125</sup>I-insulin-binding sites on target cells (Kahn *et al.*, *J. biol. Chem.*, **278**, 244; 1973; Soli *et al.*, *J. clin. Invest.*, **56**, 769; 1975). The number of binding sites increases towards normal again when serum insulin is lowered by prolonged restriction of food intake. Conversely, an increase in insulin receptors has been reported in insulin-deficient diabetic hamsters (Hepp *et al.*, *Nature*, **258**, 13; 1975). The loss of specific insulin-binding sites in hyperinsulinemic animals results from a decreased concentration

of surface insulin receptors, rather than from the blocking of receptors by endogenous insulin, the insulin-binding properties of the remaining receptors being entirely normal. Moreover, the *in vitro* exposure of cultured human lymphocytes (line IM-9) to insulin for 5-16 h decreases the concentration of insulin receptors by more than 50% (Gavin *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 84; 1974). This decrease is dependent on time, temperature, and insulin concentration. If insulin is removed from the culture medium, receptors reappear within 12-24 h and the reappearance is blocked by inhibitors of protein synthesis (Lesniak *et al.*, *Clin. Res.*, **22**, 343A, 1975). Recently, Lesniak and Roth (*J. biol. Chem.*, in the press) have demonstrated that a similar 'down regulation' of surface receptors for growth hormone (GH) occurs when IM-9 lymphocytes are exposed to human GH *in vitro*, and that insulin and GH receptors can be modulated independently on these cells. Inhibition of protein synthesis with cycloheximide produces a small but progressive loss of GH receptors which adds to the receptor loss induced by GH, suggesting that while the cycloheximide inhibits receptor synthesis, GH accelerates receptor loss. Although it has been suggested that insulin-induced receptor loss may be due to weak intrinsic proteolytic activity of insulin (Huang and Cuatrecasas, *J. biol. Chem.*, **250**, 8251; 1975), the mechanism(s) of insulin and GH-induced receptor loss remains to be established.

Another example in which a hormone induces the loss of its own surface receptors has been described by Hinkle and Tashjian (*Biochemistry*, **14**, 3845; 1975). They have shown that GH<sub>3</sub> cells (a cloned line of a rat pituitary tumour) progressively lose surface receptors for thyrotropin-releasing hormone (TRH) when they are exposed to this hypothalamic tripeptide *in vitro*. These cells, which respond to TRH by increasing prolactin secretion and synthesis, show as much as a 75% decrease in <sup>3</sup>H-TRH

binding sites after a 48-h exposure to TRH. Here again, the remaining receptors have the same affinity for TRH as controls, and receptor concentration returns to normal by 96 h after TRH removal. Loss of receptors induced by TRH, like that induced by insulin but unlike that induced by GH, is markedly inhibited by cycloheximide. In this case also, the mechanism of receptor loss is unknown and here it seems unlikely to be due to intrinsic proteolytic activity of the tripeptide hormone.

Recently, the demonstration of receptor regulation by ligands has been extended to a neurotransmitter. It has been known for some time that high concentrations of  $\beta$ -adrenergic catecholamines can induce functional desensitisation of target tissues *in vivo* and *in vitro*, but the detection of changes in the  $\beta$ -adrenergic receptors themselves was delayed by difficulties in preparing specific radiolabelled ligands. The problem of non-specific binding has now been overcome by the use of  $\beta$ -adrenergic antagonists (such as alprenolol) instead of agonists. Using  $(-)^3\text{H}$ -alprenolol as a specific ligand, Lefkowitz and his colleagues have shown that prolonged exposure of frog erythrocytes to  $\beta$ -adrenergic catecholamines *in vivo* (Mukherjee *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1945; 1975) or *in vitro* (Mickey *et al.*, *J. biol. Chem.*, **250**, 5727; 1975) leads to progressive and selective decrease (50–70%) in the number of specific alprenolol binding sites without any change in the affinity of remaining sites. Interestingly, whereas  $\beta$ -adrenergic agonists (isoproterenol > adrenaline > noradrenaline) readily modulate their receptors, the potent  $\beta$ -adrenergic antagonist, propranolol, does not; in fact, it prevents the modulation of receptors by agonists. Thus, it seems that receptor occupancy is not sufficient for modulation. The regulation of  $\beta$ -adrenergic receptors differs from that of insulin and growth hormone receptors in that treatment with cycloheximide (*in vivo*, in this case), does not prevent recovery after down regulation (Lefkowitz *et al.*, *Rec Progr. Hormone Res*, in the press), suggesting that  $\beta$ -adrenergic receptors may be reversibly inactivated rather than lost. Axelrod and his colleagues have reported similar observations on the *in vivo* regulation of  $\beta$ -adrenergic receptors of rat pineal cells (Kebabian *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3735; 1975). When these receptors are stimulated physiologically (by keeping the animals in the dark to increase the activity in sympathetic nerves supplying the pineal gland) or pharmacologically (by injecting *l*-isoproterenol), there is a rapid fall in specific  $(-)^3\text{H}$ -alprenolol binding sites

which is maximal (70% reduction) by 2 h and which begins to recover by 4 h. Exposing animals to prolonged light (which decreases sympathetic activity) increases the concentration of these receptors. In addition, they have shown that the concentration of specific alprenolol binding sites in the rat pineal normally varies with a circadian periodicity that is inversely related to the cycle of neurotransmitter release (Romero *et al.*, *Nature*, **258**, 435; 1975). Thus, there is little doubt that self regulation of neurotransmitter receptors can occur physiologically.

Teleologically, there are obvious advantages in self regulation of receptors. When cells have 'spare receptors' (that is when the maximum response occurs when only a fraction of the receptors is occupied), as is the case in most of the ligand–receptor systems discussed here, it provides an ingenious way for the cell to regulate its sensitivity to a ligand: lowering the concentration of receptor displaces the dose response to the right while still allowing the cell to make a maximal response if exposed to very high concentrations of ligand. If the cell did not have spare receptors, lowering receptor concentration would lower the maximal potential response of the cell. Just as not all surface antigens are modulated by antibody, however, so not all surface receptors are modulated by their ligand. Nor is this type of modulation the only self-regulatory receptor mechanism: a reduction in receptor affinity for its

ligand with increasing saturation of binding sites (so-called 'negative cooperativity') has been described for a number of hormones (De Meyts *et al.*, *Biochem. biophys. Res Commun.*, **55**, 154; 1973). Moreover, ligand binding is only the first step in the chain of reactions that constitute the cell's response to receptor activation, and it seems likely that regulation in response to varying concentrations of ligand can occur at many of the points in the activation sequence other than at the level of the receptor itself. For example, opiate receptor concentration is not decreased by chronic exposure to high opiate levels (Pert *et al.*, *Science*, **182**, 1359; 1973; Klee and Streaty, *Nature*, **248**, 61, 1974); such 'addicted' cells apparently adapt by increasing the concentration and/or activity of adenylate cyclase to compensate for the rapid and reversible inhibition of adenylate cyclase induced by the initial binding of opiate (Sharma *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3092; 1975). Thus, while some cases of 'desensitisation' (= 'tachyphylaxis' = 'tolerance') induced by chronic exposure to high concentrations of hormones, neurotransmitters and drugs, seem to be due to receptor modulation, others are not. In the examples discussed here, where receptor inactivation or loss is involved, the molecular mechanisms remain to be determined. With the technology now available this should not take too long. □

## Invisible astronomy

from David W. Hughes

"WHAT the eye doesn't see the heart doesn't grieve over" is a saying becoming ever less applicable to astronomers. M. E. Bailey, of the University of Sussex (page 290 of this issue of *Nature*) is not of the ranks of radio, infrared, ultraviolet, X-ray and  $\gamma$ -ray astronomers who sightlessly peruse the heavens, but of the smaller band of astronomers who predict the parameters of invisible objects by observing their effect on other stellar and planetary bodies.

One of the first successes in this field was the detection of the white dwarf companion of Sirius A by Bessel in 1844. The wavy paths of astrometric binaries on the celestial sphere has subsequently led to the discovery of planetary companions orbiting about a quarter of the nearby stars to the Sun. Bailey brings us closer to home, into our own Solar System. A great deal is known about the very small and very large objects in interplanetary space.

We observe the small meteorites (diameters normally < 1 m) by the atmospheric trails and surface impact craters they produce when they encounter the Earth. The much larger asteroids, satellites and planets, at the other extreme of the size range (diameters > 1 km) are observable through telescopes. The invisible objects in the Solar System either have sizes between these two limits (10 m < diameter < 1 km) and heliocentric distances less than 5 astronomical units, or are bigger and further away. Both are extremely difficult to detect with telescopes. But when it is considered that Pluto, the faintest planet in the Solar System, has a magnitude +14 and the 200-inch telescope at Mount Palomar, California can detect objects with magnitudes +24 (that is 10,000 times fainter) other planets must have been photographed already, their very small heliocentric velocity as yet rendering them indistinguishable from stars. The detection

of new planets in the Solar System will either be fortuitous or will have to wait until the gravitational effect they have on Pluto and Neptune produces sufficiently large perturbations to enable reasonably accurate predictions of their position to be made.

Cometary nuclei which have a predicted mass range of  $10^{18-3}$  g and a range of diameters from 1 to 100 km have not yet been directly observed. The gaseous coma which surrounds them when they are close to the Sun unfortunately shields the nucleus from observations. Due to the effects of diffraction and contrast the larger comets cannot even be seen when transiting the Sun.

Bailey now suggests a novel way of detecting interplanetary boulders and distant comets. The brightness of stars in the ecliptic plane is continuously monitored to see if the reduction of brightness produced when the star is eclipsed by a nearby interplanetary boulder or cometary nucleus can be detected. This eclipse would last for about the time it takes the object to move its own distance along its orbit; anything from 0.1 s upwards depending on its size and distance from the Earth. A conservative upper limit to the spatial density of the comet cloud, suggests that there are  $10^{10}$  comets in a belt at about 50 AU from the Sun. So using a 1-m telescope, capable of detecting 16th magnitude stars, one of the 1,000 stars visible in the field of view should suffer a short eclipse every 11 h. The problem of picking out this eclipse is an unenviable one.

Another difficulty, inherent in the proposed technique is that a boulder at 1 AU would be indistinguishable from a cometary nucleus at 100 AU. Harwit (*The Zodiacal Light and the Interplanetary medium*, NASA SP 150, 307, 1967) predicts that boulders with sizes between 10 m to 0.5 km have a spatial density of about  $2 \times 10^{-18}$  g cm $^{-3}$ . About  $10^{24}$  g of these boulders are thus distributed through a cloud  $\sim 50$  AU in radius and 2 AU thick centred on the Sun. When these get in the way of distant starlight, eclipses will also occur, adding to the number calculated by Bailey. Interestingly Sekanina (in *Asteroids, Comets and Meteoric Matter*, IAU Colloquium No. 22) proposed that the outbursts in brightness observed from comet P/Schwassmann-Wachmann I were produced by boulder impact. This comet has an orbit which always lies between that of Jupiter and Saturn. Evidence for these boulders could come from observations of their collision with other comets, Trojan asteroids or the minor satellite of Jupiter. This would require a similar experiment to that proposed by Bailey although in this case one would be looking for an increase in brightness,

not a decrease. Sekanina points out however that a  $10^8$  g boulder with a velocity of 60 km s $^{-1}$  burning out in the Earth's atmosphere would produce a fireball of magnitude +12 when observed from a place 4 AU away so again the effect is marginal.  $\square$

## Deeper insights into tRNA

from Stephen Neidle

THE transfer RNAs are molecules of central importance in protein synthesis, forming the essential link between the genetic code in the nucleic acids and developing protein molecules. Thus, the determination of the three-dimensional structure of the various tRNA species by means of X-ray crystallography, has received much attention over the past few years. In spite of considerable effort, however, only one has been both successfully crystallised and analysed—the tRNA coding for phenylalanine which has been isolated from yeast. Structural results emerged almost simultaneously from two laboratories—the MRC Laboratory of Molecular Biology, and the Massachusetts Institute of Technology (see *Nature*, **250**, 699; 1974). The Cambridge (UK) group have been investigating a monoclinic form of this tRNA, whereas the Cambridge (USA) workers have studied an orthorhombic one. These analyses have revealed the molecule as adopting very similar overall shapes in both crystallographic environments; indeed it seems likely that even the finest details of intramolecular conformation and inter-base hydrogen bonding, are very comparable. This large measure of agreement thus implies that the electron-density maps obtained have been essentially correctly interpreted by both groups.

They have now reported further analyses of these two structures, which reveal yet more detail. Indeed, so confident are they of their nucleotide-residue assignments that preliminary atomic co-ordinates have been published (Ladner *et al.*, *Nucleic Acids Res.*, **2**, 1629; 1975, and Quigley *et al.*, *Nucleic Acids Res.*, **2**, 2329; 1975). It is considered that the structures are now basically defined although they have not as yet been analysed to the limit of resolution of the diffraction data (about 2 Å), and the full armoury of structure-refinement techniques remains to be applied. The MRC group have also given an account of the 2.5 Å resolution monoclinic tRNA structure (Ladner *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4414; 1975) and the MIT workers have presented further details obtained from their 3 Å electron-

density maps (Quigley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4866; 1975). It is apparent that the two structures are virtually identical except probably at the 3'-acceptor end of the molecule; this is not surprising, as the sequence here seems to be relatively unconstrained and is able to alter its position in going from one crystallographic environment to another.

Both sets of maps are of sufficient quality for purine and pyrimidine bases to be distinguished from each other, this has also meant that the planes in which the bases lie can be more precisely defined than hitherto. Much of the intricate system of base-pairing has been confirmed, little of which is of conventional Watson-Crick type. It has also become apparent that the hydroxyl groups of many of the riboses play a very important part in helping to augment these tertiary interactions by acting as hydrogen-bond acceptors. Perhaps unexpectedly, it seems that some riboses (possibly as many as ten), adopt a C2'-endo conformation instead of the normal C3'-endo one; details of this will, however, have to await the results of the high-resolution refinements already in progress.

Several new interactions have been revealed, particularly in the neighbourhoods of the highly intricate joins and junctions between loops and stems. For example, guanine-45 seems in both models to be hydrogen-bonded to guanine-10 at the anti-codon arm-D-stem junction. Guanine-10 is itself involved in a Watson-Crick base pair with cytosine-25, thus forming a triplet of bases. Earlier interpretations of the interactions in these junctional regions had several disputed points, but these have now been largely resolved.

In retrospect, the differences and indeed difficulties in map interpretation, were not unexpected bearing in mind the great complexity of the structures, and the fact that these were the first polynucleotide structures to be examined by single crystal analysis. Among the many other points of interest which have emerged from these latest interpretations, is a neat description by the MRC group of a guanine-uracil base pair, which to date has only been observed in this yeast tRNA<sup>Phe</sup>. Such a base pair was predicted 10 years ago by Crick in his 'wobble' hypothesis, and the glycosidic bond conformational change required to accommodate the wobble seems to be as he suggested. Quigley and his colleagues have detected the probable locations of a number of metal ion sites, which are positioned to interact favourably with phosphates of the polynucleotide chain; as they suggest, such bindings may well play a part in stabilising the chain folding.

A question central to any discussion



THE annual cycle of aggressiveness now demonstrated to occur in several species of rodent (Turner and Iverson, *Ecology*, **54**, 967; 1973, Krebs and Myers, *Adv. ecol. Res.*, **8**, 238; 1974) may have an ecological and even evolutionary function of much wider significance than has hitherto been thought. The species *Clethrionomys gapperi* and *Microtus pennsylvanicus* are found in forest and grassland habitats respectively and seldom occur in the same habitat (Grant, *A. Rev. ecol. Syst.*, **3**, 79; 1972). When populations are not expanding rapidly dispersal is minimal. During periods of expansion considerable dispersal occurs resulting in invasion of normally ignored habitats Iverson and Turner reported such an event occurring when enlarged *Clethrionomys* populations invaded *Microtus* habitats (*Am. Midl. Nat.*, **88**, 440; 1972), but now it has been shown that either species can be the invader.

Working in spruce forest and old field habitats in Manitoba, Turner, Perrin and Iverson have analysed the components of the tidal effect of an invasion of a *Clethrionomys* forest by *Microtus* and shown the part played by the annual cycle of aggressiveness in returning the situation to normal (*Can. J. Zool.*, **53**, 1004;

1975). During the late autumn of 1973 a significant influx of *Microtus* into forest land was observed. Co-existence with resident *Clethrionomys* occurred throughout the winter but came to an abrupt end in May 1974. Intraspecific aggression of both species fell to its lowest during the winter, and with it the level of inter-

## Rodent aggression

from our Animal Ecology Correspondent

specific aggression. In paired encounters neither species was dominant over the other. Dominance was assessed by scoring the number of aggressive acts performed per standard encounter (*Nature*, **247**, 254; 1974). At the start of the breeding season both species became reproductively mature and both intra- and interspecific aggression increased four-fold.

Curiously enough, early in the breeding season *Microtus* became markedly dominant, winning nearly all encounters. That they did not displace the resident *Clethrionomys* is thought by Turner and his colleagues to be due to the (unspecified) advan-

tages provided by the home habitat. By early May all the infiltrators had gone leaving the forest once more to the *Clethrionomys*. It seems that co-existence is possible only when interspecific competition is at a low ebb and that it is linked to the reproductive cycle. If resource partitioning is of selective advantage in the summer, why is it not of equal importance in the winter? Food for these species is never likely to be limiting although it will fluctuate in abundance during the year, so summer food shortage is unlikely to be the wedge driving each species back to its own habitat. The sudden cease in hostilities, coinciding with the start of widespread dispersal in early autumn, means that very many more individuals survive for much longer than would be the case if temporary, out-of-range residence was not possible. This has two main effects; one is to allow emigrés to continue their search for fresh pastures, with all the evolutionary advantages this may confer (Christian, *Science*, **168**, 84; 1970); the other is to maintain a higher biomass of rodents during the winter than would otherwise be possible. This allows the existence of larger stocks of predators and a correspondingly higher secondary productivity.

of the biological significance of these structural findings is whether the conformation observed in the solid state is a representation of that existing in solution. Chen, Giege, Lord and Rich (*Biochemistry*, **14**, 4385; 1975) have attempted to answer this point by examination of the laser Raman spectra of yeast tRNA<sup>Phe</sup>. This technique has the advantage over others in that spectra can be observed in both solution and crystal; furthermore, conformational changes can often be readily detected. The observed spectra are almost identical in the two environments. Chen *et al.* also examined the spectra from both the orthorhombic crystals and a hexagonal modification, which again had essentially the same pattern of Raman frequencies and intensities. They conclude therefore that the tertiary structure of this tRNA is not only conserved in different crystal forms, but most importantly, in solution.

The next stages in the tRNA saga must be to relate this structure to its multifarious biological functions. Present attempts are necessarily speculative. Ladner *et al.* have rightly drawn attention to the quite pronounced segregation of the invariant and variable bases at several places in the crystal structure, which they suggest may be involved in synthetase recognition in one instance, and in ribosomal binding in another. It is to be hoped that the

structural studies on synthetases now in progress will answer some of these questions. Kim has also recently turned his attention to synthetase binding (*Nature*, **256**, 679; 1975) and has suggested that symmetry recognition might be important. He has observed that the tRNA crystal structure has approximate two-fold symmetry; sequence data on synthetases suggest that the subunits might also be internally organised into two domains related by two-fold symmetry. It was then straightforward to construct hypothetical symmetrical binding schemes for the two sets of domains in the two molecules; at present, these seem not implausible. □

## Making galaxies from stars

from M. G. Edmunds

GALAXIES are made of stars. Perhaps the simplest way to model the formation of a galaxy is to imagine a random distribution of stars in space, add a suitable amount of energy, and let the gravitational forces between the stars go to work. Provided not too much energy is put in, the stars will form a more spatially condensed system—a galaxy. Recently J. R. Gott (*Astrophys. J.*, **201**, 296; 1975) has described some

interesting model building of this social behaviour of stars.

Elliptical galaxies are observed to be spheroidal systems of stars which contain very little gas. Despite considerable variation in the flattening between different galaxies, the distribution of light from the galaxies as a function of distance from the centre of each system is remarkably similar. This observational relation, originally discovered by Hubble (Hubble's Other Law!), can be used to infer the radial distribution of the mass density of stars within a galaxy. Previous simple models of the gravitational contraction, or "collapse", of elliptical galaxies have given too rapid a decline of the density of stars with radius. The disagreement has led other workers to build more complicated models which introduced star formation and gas to affect the dynamics of the collapse. Gott has now resolved the discovery with observation, relying on stellar dynamics only. He assumes that small fluctuations occur in the density of a smooth Universe as it expands from the Big Bang. Stars form, and the stars in a region of high density collapse together to form a protogalaxy. The new feature of his model is a continuous increase in the mass of such a protogalaxy by the infall of stars from surrounding regions. One other process is invoked: as the Universe expands, a protogalaxy can experience gravitational tidal forces from

other protogalaxies nearby. Gott uses a very simplified model for the tidal interaction, but it nevertheless suggests two important results. The interaction will limit the final size of the galaxies and also impart angular momentum which will determine the flattening of each system. Although other models can account for the observed structure of ellipticals, Gott claims simplicity in that his models fit the data with fewer arbitrary parameters. His method is also attractive because it does not introduce any assumptions about star formation, other than that it occurs before collapse of the protogalaxy. Models which introduced star formation as a variable had to employ very *ad hoc* assumptions since no coherent theory of star formation exists.

The step from a theory of elliptical galaxy formation to a theory of spiral galaxy formation is a dangerous one. The main feature of spiral galaxies is a flat spiral disk of gas and bright young stars. A popular view, restated by Gott, is that spirals form if there is a significant amount of gas remaining in the system during the initial collapse, contrasting with the formation of ellipticals where effectively all the matter is incorporated into stars before collapse. The stars behave as in the collapse of an elliptical system, and a spheroidal distribution forms. But the gas interacts strongly with itself, rapidly dissipating much of its kinetic energy and forming a thin disk within the spheroidal "halo" of stars, rather like the meat inside a hamburger. Subsequent birth of stars from the gas gives the bright observed disk, but these stars have fairly small velocities and stay in the disk since the kinetic energy of the gas out of which they formed has been dissipated during the collapse. The halo of old stars remains.

The idea that massive haloes containing an equal or major part of the mass exist around the disks has become respectable as a result of several studies of the stability of the disks. A massive halo can stop the rapid evolution of a disk of stars into the bar-like structures which occur in numerical simulations of the stellar dynamics of disk systems. Since it is believed that spirals are not just a very transitory stage of galactic evolution, such instability of the disks is undesirable. In Gott's picture of galaxy formation the existence of such stabilising haloes around spirals is quite natural. But an attempt to determine the scale of the halo around our own Galactic disk by Maarten Schmidt (*Astrophys. J.*, **202**, 22; 1975) implies that the mass of our halo is much less than the mass of our disk. Stars in the halo possess on average much higher velocities relative to Earth than do disk stars. Schmidt uses a high velocity criterion to

identify halo members near the Sun. By making fairly reasonable assumptions about the relation between mass and luminosity for these old halo stars he infers that the local density of halo stars is at least an order of magnitude less than the mass required to stabilise the disk. The statistics of such an investigation are necessarily poor, but his result certainly indicates that mechanisms to stabilise the disk, other than the existence of a massive halo, should be sought. The result also implies that for spirals like our own Galaxy, Gott's simple stellar dynamics of spheroidal collapse which seems to work so well for ellipticals, is only a small part of the formation process. It is the material that forms the disk, rather than the halo, which will dominate the gravitational interactions during the collapse, which together with gas interactions will make a theory of spiral galaxy formation considerably more complicated. □

## Superfluid $^3\text{He}$ : an impediment removed

from P. V. E. McClintock

WITH the publication in *Physical Review Letters* (**35**, 615, 1975) of a comment by Webb, Sager and Wheatley of the University of California at La Jolla, one of the principal impediments to the identification of superfluid  $^3\text{He-B}$  as the state originally proposed by Balian and Werthamer (*Phys. Rev.*, **131**, 1553; 1963) seems to have fallen away.

Since its discovery in 1972, there has been an explosion of theoretical endeavour towards gaining a satisfactory understanding of superfluid  $^3\text{He}$  and, considering the inherent difficulties of work in the relevant temperature range below 2 mK, a quite astonishing range of experimental data has also been acquired. As a result it is now known that in a weak magnetic field there are two principal superfluid phases: the A-phase, which is stable only within a limited range of temperatures ( $T$ ) for pressures ( $P$ ) between 22 bar and the solidification pressure of 35 bar; and the B-phase, which occupies the greater part of the  $P$ - $T$  phase diagram for the liquid. Both phases exhibit complicated magnetic properties, and both are highly anisotropic, being in many respects akin to liquid crystals, with the orientations of their axes of anisotropy being determined by such factors as the applied magnetic field, thermal currents, and the proximity of the walls of the containing vessel.

The present theoretical picture of the superfluid phases, which has been constructed mainly on the basis of their observed magnetic properties,

portrays the liquid as having much in common with the superfluid electron gas in a superconducting metal, in that the superfluidity arises through the formation of pairs of particles. In contrast to the superconducting situation, however, it has been clear that the  $^3\text{He}$  atoms form pairs whose relative angular momentum is non-zero. Thus, the two atoms of a pair can be regarded as orbiting around each other somewhat like a diatomic molecule (but the analogy should not be pressed too far: unlike a diatomic molecule, the correlated pair of  $^3\text{He}$  atoms spends most of its time separated by distances very much larger than the average interatomic spacing in the liquid). Each pair in the superfluid is in the same quantum state. Thus, to change the state of one pair, for example by setting it in uniform translational motion, would involve simultaneously altering the state of every other pair which clearly is a relatively difficult thing to do, thus providing an insight into the origin of the observed superfluid properties.

Further complications arise because the  $^3\text{He}$  atomic nucleus carries a net spin and hence an associated magnetic dipole moment. Much of the theoretical effect has been aimed at discovering exactly how the orbital angular momentum of a pair is correlated with the orientations of its nuclear spins, as well as at finding the answer to the more fundamental question as to the magnitude of the orbital angular momentum itself. It became accepted at quite an early stage that the A-phase was probably in the state first suggested by Anderson and Morel (*Phys. Rev.*, **123**, 1911; 1961) and subsequently developed and justified in more detail by Anderson and Brinkman (*Phys. Rev. Lett.*, **30**, 1108; 1973). In this (ABM) state, the pair orbital angular momentum is unity, and the nuclear spins lie in the plane of the orbit either parallel ( $\uparrow\uparrow$ ) or antiparallel ( $\downarrow\downarrow$ ) to an externally applied magnetic field; there are no pairs with opposed ( $\uparrow\downarrow$ ) nuclear spins. The B-phase was identified, although with less certainty, as a form of the (BW) state originally proposed by Balian and Werthamer in which the pair angular momentum is again unity, but all three possible spin states ( $\uparrow\uparrow$ ,  $\downarrow\downarrow$ ,  $\uparrow\downarrow$ ) are present and the correlation of spins and orbit is somewhat more complicated.

Associated with both the A and B phases are characteristic frequencies  $f_A$  and  $f_B$ , dependent on  $T$  and weakly on  $P$ , which arise when the equilibrium configuration of the liquid in a magnetic field is slightly disturbed. These frequencies can be measured directly in longitudinal nuclear magnetic resonance (NMR), can be deduced from the shift of the transverse NMR frequency from its value in normal (non-

superfluid) liquid  $^3\text{He}$ , or can be measured from the magnetic ringing which follows a small incremental change in the applied magnetic field. A crucial test of the validity of the ABM and BW state assignments which was suggested by Leggett's calculations (*Phys. Rev. Lett.*, **31**, 352; 1973) involved measuring the ratio  $f_B/f_A$  at the A-B transition: according to his equations, the quantity  $(f_B/f_A) \times (\chi_B/\chi_A)$  should be precisely equal to  $(5/2)^{1/2}$ , or about 1.58. Here,  $\chi_A$ ,  $\chi_B$  are the static magnetic susceptibilities of the two phases.

Subsequent magnetic ringing experiments at La Jolla by Webb, Kleinberg and Wheatley (*Phys. Rev. Lett.*, **33**, 145; 1974) gave a value for  $f_B/f_A$  of  $1.9 \pm 0.1$  near the polycritical point (at which the A, B and normal phases are in mutual equilibrium) where  $\chi_A = \chi_B$  to an excellent approximation. This unwelcome result received support from work at Helsinki by Ahonen, Alvesalo, Haikala, Krusius and Paalanen who reported (*Phys. Lett.*, **51A**, 279; 1975) that, for similar conditions of  $P$  and  $T$ ,  $f_B/f_A = 1.93 \pm 0.05$ . These results were disconcerting in that they seemed unambiguously inconsistent with  $^3\text{He-B}$  being in the BW state, assuming that one had accepted as correct the identification of  $^3\text{He-A}$  with the ABM state.

On the other hand, Osheroff (the original discoverer of the superfluid phases during his PhD research at Cornell University) and the group at Bell Laboratories, working at the solidification pressure in a compressional cooling cell, were persistently reporting NMR results which seemed entirely consistent with the BW identification of B-phase. In particular, Osheroff (*Phys. Rev. Lett.*, **33**, 1009; 1974) found very close agreement with Leggett's  $(5/2)^{1/2}$  prediction. Thus, a situation arose where Bell Laboratories data at 35 bar seemed to support the BW state, whereas data taken at lower pressures in two other laboratories appeared to show that this state assignment was incorrect.

The overall situation was particularly disquieting because, as Leggett pointed out (*Rev. mod. Phys.*, **47**, 331; 1975) it occurred "in an area where neither theory nor experiment appears to have much room to manoeuvre". In the event, the theory and the Bell Laboratories group have stood firm and it is the experimenters at La Jolla who are doing the manoeuvring, as may be seen in the comment from Wheatley's group: their new measurements of magnetic ringing, again near the polycritical point but using a different experimental geometry, have yielded results which appear to be in reasonably good agreement with Leggett's  $(5/2)^{1/2}$  factor. It is said that a similar

## Dynamo attack

from Peter J. Smith

WHEN the principle of the self-exciting dynamo was put forward in the late 1940s to explain the origin of the Earth's magnetic field, the core motions required to maintain the dynamo were attributed to thermal convection generated by radioactive heating. Much later, Malkus (*J. geophys. Res.*, **68**, 2871; 1963) proposed alternatively that the driving force on the core could be the Earth's precession. The idea of precession-induced flow was not new, for the early dynamo theorists had considered and rejected it. What Malkus did was to show that the original reasons for rejecting a precession-driven dynamo were unsound, since which time both convection and precession have been widely accepted as serious contenders for the role of core driver.

Now, however, Rochester *et al.* (*Geophys. J.*, **43**, 661; 1975) report calculations which appear to prove that the power to be derived from precession is at least an order of magnitude too low to stir the core into stable flow. This disagreement with Malkus would be interesting in its own right; but the report is all the more remarkable in that Rochester and his colleagues go on to criticise Malkus severely not only for his result but for the way he obtained it. Specifically, they accuse Malkus of appealing to dubious analogies, of claiming agreement with previous work where no such

agreement exists, of publishing inconsistent equations, of error in mathematical logic, of mathematical oversimplification and of numerical errors, among other things. They further object that in later articles Malkus has not only ignored the few published objections to his 1963 work, but has repeated the errors and retracted nothing.

This is the most severe attack to have appeared in the Earth sciences for many years and raises ghosts from another century. But whether or not one agrees with the form of the criticism, it would be a pity to overlook the serious issue it raises. Many people have come to believe in the feasibility of a precessionary dynamo because they have read Malkus's conclusion but not the arguments on which it is based. The problem is that studies in this field are so esoteric that only a handful of people in the world can understand them; indeed, even Rochester and his colleagues claim not to be competent to deal with all the points involved. As they themselves point out, under such circumstances the rationale for avoiding unpleasant criticism, as described by Ravetz (*Scientific Knowledge and its Social Problems*, OUP, 1971), breaks down and myths develop.

The question now is: is the precessionary dynamo such a myth or not? For the irony is that most Earth scientists will find the detailed arguments of Rochester *et al.* no easier to understand than those of Malkus, and will, as before, have to be content with the conclusion.

manoeuvre has also been carried out in Helsinki. The reason for the earlier discrepant results is not yet entirely clear, but they can perhaps be regarded as a reminder of how much is still not properly understood about the liquid.

The new measurements at La Jolla, which provide a welcome vindication for the work of Osheroff and the Bell Laboratories' group, may be regarded as greatly strengthening the present state identifications of both superfluid phases of liquid  $^3\text{He}$  and will, no doubt, have been received with sighs of relief from the many theorists working in the field □

## Regulatory genes and quantum evolution

from A. Hallam

THE abrupt appearance of higher taxa in the fossil record has long been something of an enigma to palaeontologists. Although it is true that temporal sequences of fossils provide some of the

best evidence of evolution, Darwin himself was somewhat embarrassed by the sparsity of transitional forms, which he attempted to explain away by invoking numerous erosional breaks in the sequence of strata bearing the fossils. Since Darwin, many of these 'stratigraphic' gaps have been filled as a result of research throughout the world by numerous people, yet the quantum jumps between phyla and lower taxa by and large remain. A few decades ago Simpson made a valiant attempt to grapple with the problem by putting forward an ecological model of 'quantum evolution' consistent with current genetic theory. This involved small populations of particular species crossing some environmental threshold into a new adaptive zone (certain mammals re-entering the sea for example), whereupon there ensued rapid evolutionary radiation and hence pronounced morphological divergence from the parent stock.

Valuable as Simpson's ideas have proved, major problems and uncertain-

ties have remained about the underlying genetic control, and further attempts must be made to square the facts of palaeontology with the new knowledge and insights gained from molecular biology. Evolutionary genetics has been primarily concerned with structural genes, those genes which are transcribed into RNA, leading to the synthesis of particular proteins and enzymes. It turns out however that there are striking similarities in the basic proteins and enzyme activities throughout the organic world, from primitive prokaryotes to mammals, which vastly outweigh the differences. The enormous differences within the animal and plant kingdoms are much more a matter of organisation and structure. This suggests to Valentine and Campbell (*Am. Sci.*, **63**, 673; 1975) that the evolutionary divergence of major and perhaps even minor taxa has been effected mainly by changing patterns of gene regulation rather than the mere substitution of amino acid sequences in proteins.

The regulatory model proposed for prokaryotes (bacteria and blue-green algae) by Jacob and Monod, involving operator and repressor genes, has been verified in many particulars, but gene regulation in the much more complex eukaryotes (the rest of the organic world) is far less well understood. Valentine and Campbell favour the general regulatory model for higher organisms put forward by Britten and Davidson (*Q. Rev. Biol.*, **46**, 111; 1971). 'Sensor' genes receive a stimulus, probably hormonal, and cause 'integrator' genes to transcribe. The transcription product, activator RNA, is detected by 'receptor' genes, which in turn cause structural genes to transcribe. Whole batteries of receptors, which need not be mutually exclusive, operate under the control of a single integrator, and a hierarchy of sets could be controlled by a series of master sensors. It is evident that mutations affecting regulatory genes, together with chromosomal rearrangements, could create in a geologically short time a wide variety of novel patterns involving large numbers of structural genes.

In broad outline evolution can be envisaged to have proceeded in a number of major steps, each involving a repatterning of genes in new combinations and expansion of the regulatory apparatus, as cell types differentiated and complex body-layer and organ systems progressively developed. Thus for the animal kingdom the sequence is: protozoans; diploblastic metazoans (sponges, jellyfish); triploblastic metazoans (flatworms); primitive burrowing coelomates (many worms); advanced coelomates, often with skeletons. Each successive step involved significant addition of new in-



## A hundred years ago

OUR readers no doubt know that we have a younger French sister who appears under the name of *La Nature*. We have just received from Germany a specimen of another of the family, rejoicing in the name of *Die Natur*. This seems, however, to be a new series of an old-established journal, but whether it has always appeared under its present name we cannot make out. It is conducted by Dr. Otto Ule and Dr. Karl Muller, of Halle, is mainly devoted to natural history, and the number sent us contains several interesting articles; among these is one on the African Steppes, by Dr. Ule. from *Nature*, 13, January 27, 256; 1876

formation to the regulatory apparatus and creation of batteries of structural genes in new combinations. The fossil record indicates that all the steps were accomplished by the end of the Cambrian, some 500 million years ago, when all but two of the living phyla that are well skeletonised had already appeared. This does not mean that most palaeontologists are necessarily only concerned with the equivalent of philosophy's 'footnotes to Plato', because there is still a vast amount to be discovered about the detailed patterns of evolution as revealed by fossil distributions in time and space. The Valentine and Campbell paper is valuable not so much in prediction as in providing a satisfying interpretive framework for further research. □

## Rapid quenching: second harvest

from Robert W. Cahn

The Second International Conference on Rapidly Quenched Metals was held at the Massachusetts Institute of Technology on November 17-19, 1975. It was organised by Professor N. J. Grant, of MIT, and Professor B. C. Giessen, of Northeastern University.

WHEN a canny farmer ploughs a new field, the first harvest will as likely as not be bounteous; but the second harvest defines the long-term value of the land. The Conference was dedicated to Pol Duwez, the American metallurgist, who first opened up the field of

rapidly quenched metals 15 years ago; a rich first harvest was reaped in 1970 during the First Conference, and now the second has proved that he made a sound investment.

The Conference was immediately followed by a Workshop (held at Northeastern University, Boston) devoted to the applications of amorphous alloys. These materials—also known as metallic glasses—are merely one subcategory of rapidly quenched metals, but the fact that two days were entirely devoted to them reflects the organisers' judgment, vindicated by the Conference and Workshop, that the corn now grows tallest in this corner of the field.

The first Conference, in 1970, was largely concerned with extended solid solubility and with anomalous intermetallic phases resulting from splat-quenching of alloy melts. Fewer papers this year dealt with these concerns than one would have foretold then. Some of these were concerned with steels: groups at Cambridge (UK), Tohoku University (Japan), and the University of Pennsylvania (USA) examined the behaviour of splat-quenched steels, both in their initial state and after subsequent transformation: crystallography, morphology and kinetics were all investigated. The Cambridge group concentrated on familiar steels such as Fe-Mo-C steels, and observed solubility extension in ferrite, retained austenite and a number of unfamiliar carbides: the steels were at all stages crystalline. The others examined Fe-P-C, one of the transition metal/metalloid combinations which become amorphous on quenching, and investigated the details of subsequent crystallisation. This is an area where much more research is now needed, with good prospects of industrial applications.

Aluminium alloys continue to be the favourites among those who prefer to investigate crystalline forms of metastability. Some of these have reached the verge of industrial use. A group at the Battelle Institute in Frankfurt (Germany) have scaled up a technique for splat-quenching and compaction of Al-Fe alloys (for some years now the preferred alloys for practical application), further alloyed with Mg, Mn and Cr. The product is not only very strong, even at 300 °C, but—like a number of other chromium-bearing splat-quenched alloys—extremely resistant to corrosion: there was no detectable attack by superheated seawater at 150 °C. Some applications are immediately apparent. A group from the University of Sussex (UK) showed how a plasma-spraying gun, with improvements to help keep the deposit cool, can be used to spray highly super-saturated and thus extremely strong Al-Cu alloy sheets.

An impressive paper from Battelle



Pacific Northwest (USA) showed how a large sputter-deposition apparatus can be used to make sizeable artefacts of metastable materials, including alloy glasses. This seems to be the only available way of making such objects other than in thin-sheet form.

Surprisingly little attention was paid to the regularities governing solubility extension and metastable intermetallic phases: one paper from Northeastern University identified some relationships between stable and metastable ordered intermediate phases, and a major paper from Carnegie-Mellon University (USA) pinpointed the influence of 'electronic factors' à la Hume-Rothery on the extension of solid solubility in various alloy systems. In summary, unfavourable size factors can be overcome by rapid freezing but unfavourable electronic factors cannot.

Over the past five years there have been notable developments in quenching techniques. A good deal of interest was manifested in the technique of plasma-spraying. The high point, however, was a paper from the Battelle Laboratories in Ohio (USA) describing a patented new method for making rapidly quenched ribbons. A rapidly rotating contoured wheel dips into a metallic melt, either subjacent in a crucible or pendant over the wheel from a rod of solid metal: the wheel 'spins' out a ribbon which lifts clear after it has frozen. The ribbon can either be spooled or else the wheel can be provided with a series of circumferential nicks; when that is done, a shower of short filament lengths is generated, ready to substitute for chopped fibre in (for instance) reinforced concrete. This technique has already been licensed and is soon to go into industrial production. Though the method has required a good deal of fine tuning it has the essential simplicity of all classic production techniques and will be a significant rival to the roller-casting technique, already in commercial use by Allied Chemical Corporation to make their 'Metglas' amorphous alloys. That Corporation's scientists were much in evidence during the five days, and a number of new compositions were announced, in particular Metglas 2605 ( $\text{Fe}_{80}\text{B}_{20}$ ), notable for high magnetic permeability and high strength, and a range of Be-Ti-Zr glasses such as the high-strength Metglas 0422,  $\text{Be}_{40}\text{Ti}_{50}\text{Zr}_{10}$ .

Much attention was devoted to the structure of amorphous alloys (a piquant concept, since 'amorphous' derives from the Green root for 'formless'). Several laboratories, notably the Tohoku group, the Technical University of Berlin and the University of Paris-Sud, have firmly established by X-ray and neutron diffraction and other methods that alloy glasses have

very pronounced compositional short-range order, as had been suspected. The interest evident five years ago in computer-modelling of alloy glasses based on 'dense random packing' (with a view to comparing computed and experimentally derived pair distribution functions) has been fully maintained, while the rather pointless semantic controversy between the 'true amorphous' and 'microcrystalline' models shows signs of attenuating. In this field of research, scientists from the IBM Watson Research Centre at Yorktown Heights (USA) and the long established group at Harvard are making major contributions.

More than half of the Conference/Workshop was devoted to the mechanical and magnetic properties of alloy glasses. The combination of very high strength with appreciable ductility of these materials has been well documented, and no surprises emerged. Interest is beginning to focus on the influence of annealing, below or above the crystallisation temperature, on mechanical behaviour. One intriguing observation was that annealing can embrittle phosphorus-bearing, deformed, alloy glasses, and controversial evidence suggests that phosphorus diffuses to shear bands and promotes fracture.

The new Be-Ti-Zr ribbons will be of particular interest. One novelty was the observation that fracture toughness is a sensitive function of ribbon thickness, and is an optimum for a thickness less than the maximum which can be fabricated. This is not as yet understood. Although the makers of 'Metglas' ribbons have for some time now emphasised the role of their product as a reinforcing fibre, this Conference heard the very first public account of a systematic attempt to put this claim into effect. A group at Pratt & Whitney Aircraft experimented with epoxy resins reinforced with one of the commercial ribbons, up to 40% volume fraction; though the resin-ribbon bond was weak, good reinforcement was attained both longitudinally and transversely. It is astonishing that this should be the first published investigation of its kind, and that no accounts of filament-winding have yet appeared.

The topic which now excites the greatest interest, both scientifically and industrially is the ferromagnetic behaviour of various amorphous alloys based on iron and nickel, and to a lesser extent cobalt. A great many contributions centred upon this, especially an impressive cluster from Bell Telephone Laboratories (Murray Hill, USA) and another from the General Electric R and D Centre at Schenectady (USA). An academic group at the University of Pennsylvania and the

extremely energetic Japanese group at Tohoku University are also active in this field. Increasingly, the various groups are manufacturing their own glassy materials. (If two individuals may be picked out for sheer collaborative productivity in research on amorphous alloys, honours should go to H. S. Chen of Bell Telephone and T. Masumoto of Tohoku).

The major group of alloy glasses—including now the new commercial  $\text{Fe}_{80}\text{B}_{20}$ —has high permeability, low coercivity and reasonably good performance at high frequencies. Materials with zero magnetostriction and consequently extremely low anisotropy energies and high permeabilities, are now routine. A wide range of magnetic properties is in fact attainable in one and the same alloy, merely by changing the details of the magnetic annealing treatment.

Magnetic domains are detected in spite of ultra-low anisotropies and the ribbons are sensitive to handling, though less so than materials such as crystalline permalloys (as one exasperated scientist remarked, one dare not look at permalloy for fear of spoiling its permeability!) Roller-cast ribbons have periodic variations of magnetic behaviour, related to periodic variations of internal stress introduced during production. The intimate symbiosis between minutiae of manufacture and magnetic behaviour, long familiar in the world of magnetic materials, is again very much in evidence. A newly discovered family of glassy alloys based on Fe/Th compositions and made by massive sputter-deposition, prove to be strong permanent magnets. Here high anisotropy appears to stem from local variations in composition and structural order.

As the GE group in particular indicated, there are in principle very extensive magnetic applications for alloy glasses in industry, especially the high-permeability kind. Filter chokes, transducers, loading coils, fluxmeters, magnetic amplifiers, L-F inverters and indeed transformers are all candidates. Much of the ground work in establishing suitable compositions and forms of magnetic annealing has been done. There is just one remaining obstacle, but it is a major one! Up to the present, alloy glass ribbons can only be made a few millimetres wide. For most applications, wide sheet is essential. The commercial people present were extremely tight-lipped about this crucial problem, which seems to be very obstinate. A very large prize, indeed, awaits the processing specialist who is first able to resolve this impasse, either by inventing a new form of continuous quench-casting or by some totally different approach. □

# review article

## Differential function of major histocompatibility complex antigens in T-lymphocyte activation

Fritz H. Bach, Marilyn L. Bach & Paul M. Sondel

*The antigenic systems of the major histocompatibility complex can be subdivided into those which are serologically detectable and those which are detected in tests with mixed lymphocytes. The two systems have different roles in the activation of separate populations of T lymphocytes.*

FEW investigators attracted to the study of transplantation antigens controlled by the major histocompatibility complex (MHC) foresaw the multitude of fascinating biological phenomena related to genes of this region. MHC loci are involved not only in the control of strong transplantation antigens, but also in control of immune response, disease susceptibility, immune cell interactions, level of serum complement components and perhaps in developmental abnormalities; it seems that many of the genes determine cell surface structures. This review will deal largely with antigens controlled by the MHC, their genetic control and function in immunological reactions.

MHC associated antigens were originally detected by transplantation *in vivo*; the recognition of serologically defined antigens controlled by genes of that same genetic complex led to the question whether these antigens were themselves the strong histocompatibility (transplantation) antigens or genetic markers for them. Additional serological studies as well as the use of two *in vitro* models of cell-mediated immunity have provided evidence for the existence of cell surface antigens determined by MHC genes other than those controlling the initially described serologically defined antigens. The accumulated data suggest that different antigens of the MHC are separable on the basis not only of the recombinational events separating the genes coding for them, but also of different functional roles in terms of lymphocyte subpopulations that respond to them.

### Detection of MHC antigens

The two *in vitro* cellular models that have most extensively been used for the analysis of MHC associated antigens are the mixed leukocyte culture (MLC)<sup>1,2</sup> and cell-mediated lympholysis (CML)<sup>3-6</sup> assays (Fig. 1). In MLC tests, lymphocytes from one individual (the responder) are cultured for 4-7 d with stimulating lymphocytes from a second individual. To prevent their proliferation, stimulating cells are treated with mitomycin-C (ref. 7) or X rays<sup>8</sup> before they are added to responding cells. When the stimulating cells are from individuals whose MHC is different from that of the responder the untreated responder lymphocytes proliferate; this is detected by incorporation

of tritiated thymidine (<sup>3</sup>H-TdR) into the proliferating cells in the MLC assay. For CML assays, cytotoxic T lymphocytes (CTLs) are "generated" in mixed leukocyte cultures<sup>9-11</sup>. After incubation for several days, the responding cells are assayed as effector lymphocytes for their cytolytic activity as measured by their ability to lyse radioactively sodium chromate (<sup>51</sup>Cr) labelled target cells; if the target cells carry the same (or cross reactive) antigens as the stimulating (sensitising) cells in MLC, then the target cells are lysed<sup>12</sup>. The amount of <sup>51</sup>Cr released into the medium is a quantitative assay for lysis and is expressed as CML. Since the terminology in this field is complex and can be difficult to follow, terms and abbreviations used in this article are listed in Table 1.

On the basis of serological findings and the use of MLC and CML tests, it is possible to divide MHC determined cell surface antigens into at least three categories. First, the classical serologically defined antigens that are present on the surface of essentially all cells. We shall refer to these as the SD antigens. Second, some antigens which can be detected serologically are present only on restricted cell types, among them B lymphocytes, macrophages, epidermal cells and sperm<sup>13</sup>. These antigens are referred to as Ia (Ir-associated) in mouse and by other designations in man. Although it has not been conclusively established that these mouse and human antigenic systems are homologous we shall make that assumption and, since the human terminology has not been unified, refer to both as Ia. Third, there are the MHC antigens that, if different in two individuals, lead to an MLC proliferative response<sup>14,15</sup>. We shall refer to these as LD antigens<sup>15,16</sup>. Whereas genes determining the MHC SD antigens are genetically separable from those determining the Ia and LD antigens in both mouse and man, there may be some degree of identity between Ia and LD determinants.

It must be stressed that the designations SD, Ia and LD are simply terms we use to distinguish between MHC determinants that may have different biological roles. Since the terminology for these antigens and the loci coding for them are different in the several experimental species used for various studies, the terms allow simplified reference to and discussion of pertinent phenomenology related to what are presumably homologous systems in several species. In addition, in a single species several genetically separable loci coding for antigens all apparently subserving a single function can be conveniently referred to with a single

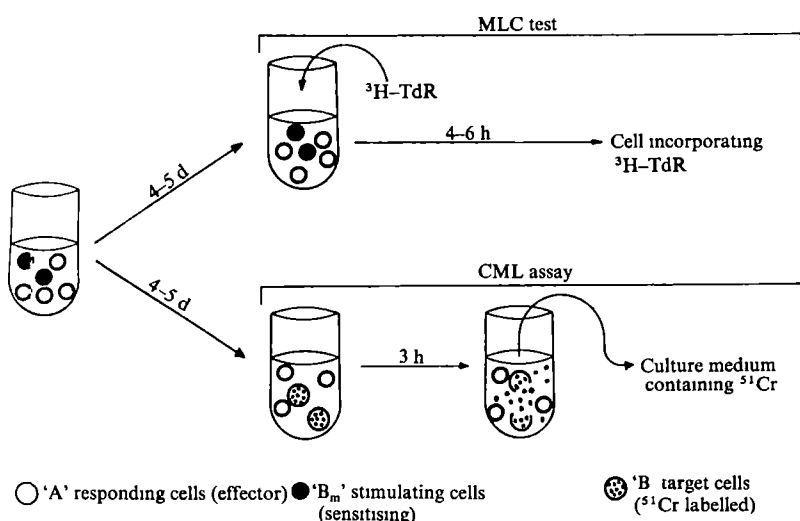


Fig. 1 Schematic representation of the MLC and CML tests. Cells differing for the major histocompatibility complex are mixed *in vitro*: for the MLC assay, proliferative activity is assayed after 4-5 d by studying incorporation of radioactive thymidine; for the CML assay, the initial responding cells are tested for their cytotoxic capacity against radioactively labelled target cells, the amount of radioactive label released from the target cells by the effector cells is expressed as % CML.

term. The terms should in no way imply that a function associated with LD cannot also be associated with SD; for example, that SD antigens cannot induce lymphocyte proliferation. On the premise that the broad biological principles discussed in this article are the same in mouse and man, we shall draw freely on data obtained in both species.

## Genetics of the MHC in mouse and man

The MHC in mouse, called *H-2*, has been divided into five regions<sup>13,17</sup>, K, I, S, G and D on the basis of marker loci for each region (Fig. 2). The K and D regions have as their marker loci *H-2K* and *H-2D*, that control the *H-2* SD antigens. The I region can be subdivided on the basis of different Ia determinants associated with each of three sub-regions: I-A, I-B and I-C<sup>13,14,17,18</sup>. Included in I-A are loci determining certain Ia antigens, an immune response (*Ir*) locus controlling the ability of an animal to respond immunologically to a variety of antigens, and the strongest *H-2* LD locus, that is differences for this locus lead to strong proliferative responses in MLC. Within this subregion the genes determining Ia antigens, LD determinants, and controlling immune response have not been genetically

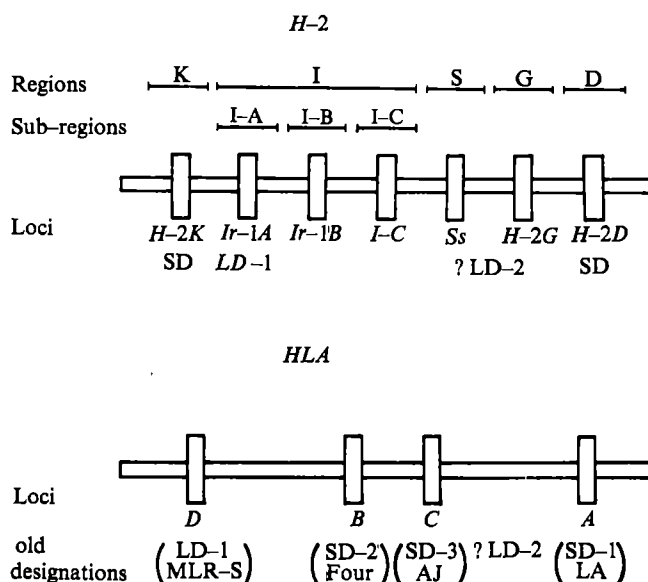
separated. Partial identity of *Ir* and Ia genes is suggested by the existence of a soluble factor that bears Ia antigen determinants and that is involved in regulation of the immune response<sup>19,20</sup>.

The human MHC, termed HLA (Fig. 2) has three different SD loci, *HLA-A*, *HLA-B* and *HLA-C* and one strong LD locus, *HLA-D*; a weak LD locus may exist between *HLA-A* and *HLA-C*. Van Leeuwen *et al.*<sup>21</sup> and more recently Winchester *et al.*<sup>22</sup> have demonstrated the existence of cell surface antigens determined by genes of the human MHC that at least in part have a similar tissue distribution to the mouse Ia antigens.

For convenience in further discussion, the word "locus" will be used to describe the genetic control of all antigens or determinants where it has not been shown that more than one cistron is involved. Two strains (individuals) designated "LD different" differ for the strong LD locus and are identical for the SD loci; those designated "SD different" differ for either the K and/or D regions in mouse or the SD loci in man but are identical for the strong LD

Fig. 2 Diagrammatic representation of the major histocompatibility complex in mouse and man. In mouse, the complex is divided into five regions with the I region further divided into three sub-regions. Marker loci for each of the sub-regions are shown. The strongest LD locus is in the I-A sub-region; a weaker LD locus may be present between *Ss* and *H-2D*. The *H-2D* and *H-2K* loci are the SD loci.

Table 1 Abbreviations and terms	
MHC	Major histocompatibility complex, <i>H-2</i> in mouse and <i>HLA</i> in man.
<i>Ir</i>	Immune response loci alleles which control the ability of an animal to respond to a variety of antigens.
Ia	The antigens controlled by the <i>H-2</i> I region in mouse that are primarily expressed on B lymphocytes, macrophages, epidermal cells and sperm. An apparently homologous antigenic system has been described in man.
MLC	Mixed leukocyte culture test; usually assayed by study of thymidine incorporation into stimulated lymphocytes.
LD	The MHC loci and antigens primarily responsible for inducing thymidine incorporation (cell proliferation) in MLC. The stronger of them are controlled by <i>HLA-D</i> in man and <i>LD-1</i> in mouse.
PHCs	Proliferating helper cells; the T lymphocyte subpopulation that is primarily responsive to MHC LD antigens. These T cells do not adhere well to allogeneic monolayers and carry the Ly-1 differentiation antigens.
CML	Cell-mediated lympholysis assay, assayed by studying release of <sup>51</sup> Cr from labelled target cells.
SD	The MHC loci and antigens (or genetic markers for the antigens) that serve as the primary targets for the cytotoxic T lymphocytes. These are the classical serologically defined antigens of <i>H-2K</i> and <i>H-2D</i> in mouse and <i>HLA-A</i> , <i>-B</i> and <i>-C</i> in man.
CTLs	Cytotoxic T lymphocytes active in the CML assay. These T cells adhere to allogeneic monolayers and carry the Ly-2 and Ly-3 differentiation antigens.



locus. This focuses on only the MHC LD and SD loci; other MHC loci may well be involved in the reactions discussed.

## Genetic control of MLC

In man, the *HLA-D* (*LD-1*) locus has the predominant role in inducing proliferation in MLC; differences for the SD loci do not, by themselves, lead to strong MLC activation (reviewed in refs 23 and 24). Cells of siblings that are *HLA-D* identical but differ for SD loci either do not stimulate each other at all or do only minimally<sup>25,26</sup>; this low stimulation may be due to SD antigenic differences themselves and/or to a weak LD locus between *HLA-A* and *HLA-C*. It is not established whether *HLA* SD antigens provide a significant additional proliferative stimulus in MLC in the presence of *HLA-D* disparity.

In mouse, a strong LD locus in the *H-2* I region is primarily responsible for proliferative events in MLC; differences for the *H-2* SD regions (K and/or D) given I region identity are also significantly stimulatory although to a lesser extent. These latter proliferative responses, as in man, may be caused by the SD antigens or by LD-type stimuli within the K and D regions (for example, there is evidence for an LD locus between S and D<sup>27</sup>). In mouse, apparently unlike man, there is a locus (*Mls*) alleles of which segregate independently of *H-2*, and differences at which lead to an MLC proliferative response<sup>28</sup>. Even in xenogeneic combinations, such as man-mouse, the same *H-2* regions that lead to an allogeneic MLC reaction provide the proliferative stimulus (K. F. Lindahl and F.H.B., in preparation).

## Genetic control of CML

Despite the finding that the MHC LD antigens are primarily responsible for activating the proliferative response in MLC, and that cytotoxic T lymphocytes (CTLs) active in CML are generated in MLC, the target antigens primarily recognised by CTLs are not LD. Rather the specific lytic effect of CTLs is directed at either the MHC SD antigens themselves or the phenotypic product of genes very closely linked to those determining the SD antigens<sup>29-33</sup>. This is, once again, true even in xenogeneic combinations<sup>34</sup>. We shall, for simplicity, refer to them as the SD antigens.

These findings are most elegantly demonstrated in "three-cell" experiments<sup>35,36</sup>, in which cells of one strain (individual) are simultaneously stimulated by mitomycin-C treated cells of two other strains, one differing from the responding cells by LD and the second differing by SD antigens (Table 2). In addition these data give evidence for "LD-SD collaboration". Cells of two LD different strains (AQR-B10.T(6R)) stimulate each other significantly in MLC but the LD stimulus by itself does not generate cytotoxic cells directed at the SD target. Two SD region different strains (AQR-B10.A) lead to a relatively weaker proliferative response (compared with that caused by I region LD differences) and highly significant cytotoxicity against B10.A<sup>37,38</sup>. The simultaneous presentation of LD and SD stimuli, however, leads to a markedly enhanced cytotoxic response against SD different target cells (LD-SD collaboration). The CML % is linearly related to the log of the number of CTLs; a difference from 21.8% to 40.4% thus represents much greater than a twofold difference in terms of lytic potential<sup>32</sup>. The LD-SD collaboration will be analysed in greater detail later (see Table 4). It should be pointed out, however, that whereas the stimulation of AQR cells by B10.T(6R) does not lead to CML directed at B10.A, relatively low level but highly significant CML directed at B10.T(6R) does result<sup>32,38</sup> (see also Table 4).

These results demonstrate clearly the LD-SD co-operation; that is the presence of an LD difference in the stimulating mixture markedly enhances the development

Table 2 LD-SD collaboration in a three-cell experiment

<sup>3</sup> H-TdR incorporated (c.p.m.) $\pm$ s.d.*	Target cell	%CML $\pm$ s.d.
10,842 $\pm$ 236 AQR + 6R <sub>m</sub>	B10.A	-1.2 $\pm$ 3.1
4,113 $\pm$ 190 AQR + B10.A <sub>m</sub>	B10.A	21.8 $\pm$ 2.1
8,292 $\pm$ 309 AQR + 6R <sub>m</sub> + B10.A <sub>m</sub>	B10.A	40.4 $\pm$ 2.8

\*The control culture AQR(AQR)<sub>m</sub> incorporated 2,926  $\pm$  254 c.p.m. <sup>3</sup>H-TdR.

The strain B10.T(6R) is abbreviated as 6R. The designations for the seven regions and subregions of *H-2* (K, I-A, I-B, I-C, S, G and D) for the strains are as follows: AQR, qkkddddd; 6R, qqqqq?d; B10.A, kkkddddd. As such, AQR and 6R are *H-2* SD identical but different by LD, and AQR and B10.A are K region different but identical for the rest of *H-2* (D. J. Schendel and F.H.B., unpublished data).

of cytotoxicity against the SD region target even though the LD stimulus by itself does not generate cytotoxic cells against that target. In contrast, the presence of the SD antigen does not enhance anti-LD (anti-I region) CML (see Table 4 and later discussion).

Experiments with human cells yield similar results with one exception: SD antigenic disparity "alone" (that is *HLA-D* identity) leads neither to a strong proliferative response nor to a detectable cytotoxic response<sup>25,29,30,36,39</sup> contrasting with the MLC and CML associated with SD region differences in mouse. The high magnitude proliferative and cytotoxic responses seen in LD plus SD disparate human MLCs would suggest that this difference is not due to insensitivity of the human method although the culture technique cannot be ruled out as the explanation. The discrepancy could be based on variation of human and mouse SD antigen presentation or recognition. Alternatively, it may be explained by the evolutionary generation of somewhat different arrangements of the genes of human and mouse MHCs (see Fig. 1). In mouse, the *H-2K* locus and loci of the I-A sub-region are very closely linked; recombinational events between them may in most cases result in the inclusion of some relatively weak LD loci in what we call the "SD region". In contrast the probably tenfold greater recombinational frequency between *HLA-B* and *HLA-D* may prevent such LD loci from being included in the SD segment of most human recombinant chromosomes, thereby allowing a more complete genetic separation of LD and SD antigens in human recombinants.

## Cellular recognition of MHC antigens

The cells responding in MLC and CML are primarily T cells. On the basis of evidence that functionally different subpopulations of T cells exist<sup>40</sup>, we suggested in 1972<sup>41</sup> that the LD-SD dichotomy might be explained at the cellular level by the existence of two separate subpopulations of T cells, one responding primarily to LD (the proliferating helper cell—PHC) and another responding to SD (the cytotoxic T lymphocyte—CTL). Results of experiments using cellular immunoadsorbents<sup>42</sup> (monolayers of allogeneic adherent cells) were consistent with the existence of two separate populations of T lymphocytes, one responding to LD by proliferation and not adhering to an allogeneic monolayer; the other, the CTLs adhering to the monolayer. These two cell populations can be separated before sensitisation in MLC. An example of such an experiment is shown in Fig. 3. Cells of individual A are adsorbed on to either an A, B or C monolayer; 1 h later the non-adherent cells are removed from the monolayer and stimulated with cells of individual B and tested on B target cells. The level of cytotoxicity generated against B when the non-adherent A cells recovered from the B monolayer are stimulated with B<sub>m</sub> is minimal compared with similarly stimulated non-adherent cells from the A or C monolayers. In 12 experiments<sup>43</sup> the average reduction in cytotoxic activity



(lytic units or potency) of non-adherent lymphocytes after pre-adsorption on allogeneic monolayers was 70% whereas only a 12% reduction in  $^3\text{H}$ -TdR incorporation measured in MLC was observed. The adsorption experiments are consistent with the finding that the CTLs divide (ref. 43 and B. J. Alter and F.H.B., unpublished) (incorporate  $^3\text{H}$ -TdR), although they seem to contribute only a relatively small percentage to the total  $^3\text{H}$ -TdR incorporated in a normal MLC—most is incorporated by the PHCs<sup>41,42</sup>.

The concept of two cell populations responding to LD and SD antigens was strengthened by the findings of Cantor and Boyse<sup>44,45</sup> using antisera against Ly cell surface antigens. These antigens are T-cell differentiation markers controlled by three loci, *Ly-1*, *Ly-2* and *Ly-3*. It seems

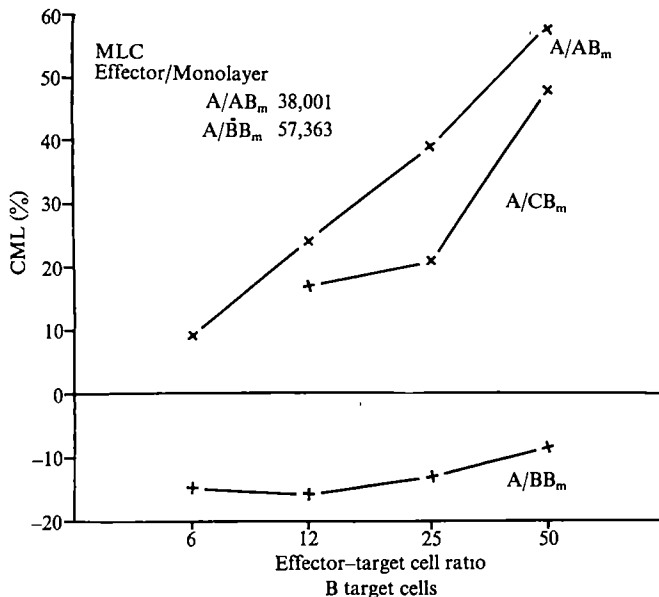


Fig. 3 Non-sensitised reciprocal adsorption. Cells of individual A (listed before the /) are adsorbed on either an autologous, A, monolayer or on allogeneic monolayers from individuals B or C (listed immediately after the /). The non-adherent cells from the monolayer are then stimulated with mitomycin-C treated cells from individual B (designated B<sub>m</sub>), and assayed 5 d later for their ability to lyse B target cells. The marked reduction in cytotoxic activity following adsorption on the B monolayer, compared with the A or the C monolayer, is shown with no significant decrease in the proliferative activity. The reciprocal experiment stimulating the non-adherent cells with mitomycin-C treated cells of individual C and testing on C target cells in CML gave qualitatively similar results. (From K. S. Zier and F.H.B., unpublished.)

that LD responsive PHCs are *Ly-1*+ and do not mediate cytotoxicity; *Ly-2,3*+ cells divide and become CTLs. Support for the dual T-cell concept has also come from studies of Wagner *et al.*<sup>46,47</sup>.

## Responses to LD and SD *in vivo*

The functional distinction of LD and SD antigens has been supported largely by experiments involving discrimination of cellular immune recognition *in vitro*. Of great importance is the integration of both antigenic and cellular distinctions into the overall process of allograft reactions *in vivo*. The importance of LD and SD antigens has been studied *in vivo* using both graft versus host and graft rejection assays.

Essentially similar results have been obtained in a number of graft versus host (GvH) test systems including splenomegaly, lymph node enlargement, or increased radioactive IUdR incorporation into spleen or lymph node cells following injection of histoincompatible immunocompetent cells. Results closely parallel those found in the MLC; that is

LD differences are very potent at eliciting GvH reactions, the SD differences are relatively weak in this regard<sup>48-50</sup>. Representative results from one experiment performed by Elkins *et al.*<sup>50</sup> are given in Table 3. Most important are the findings that the LD differences (I+S) are quite strong in eliciting the response; two strains that differ by only the K region or the D region plus I-B, I-C, S and G do not elicit a significant response in this test system<sup>49</sup>.

In man, the survival of transplanted tissue in non-presensitised individuals is prolonged by identity for either LD or SD antigens<sup>51-53</sup>. Of the two types of MHC determinants, preliminary data in man suggest that identity for LD has greater predictive significance for the length of graft survival, at least with respect to skin and kidney grafts<sup>51</sup>; but this distinction requires further study.

One important facet of the response to allogeneic tissue *in vivo* is not usually detected by routine MLC and CML testing *in vitro*: the influence of alloantibody. Human renal graft recipients immunised with multiple whole blood transfusions often produce cytotoxic alloantibody. The presence of antibody directed against donor tissue SD antigens induces accelerated and sometimes hyperacute

Table 3a Response of B10.T(6R) thymocytes to I+S region difference

Host strain	% activity recovered in spleen*	H-2 region difference
(AQR × 6R) F <sub>1</sub>	0.63 (0.44-0.73)	I+S
B10.A	1.12 (1.10-1.44)	K+I+S
B10.T(6R)	0.03 (0.02-0.03)	None

\*Median (range)

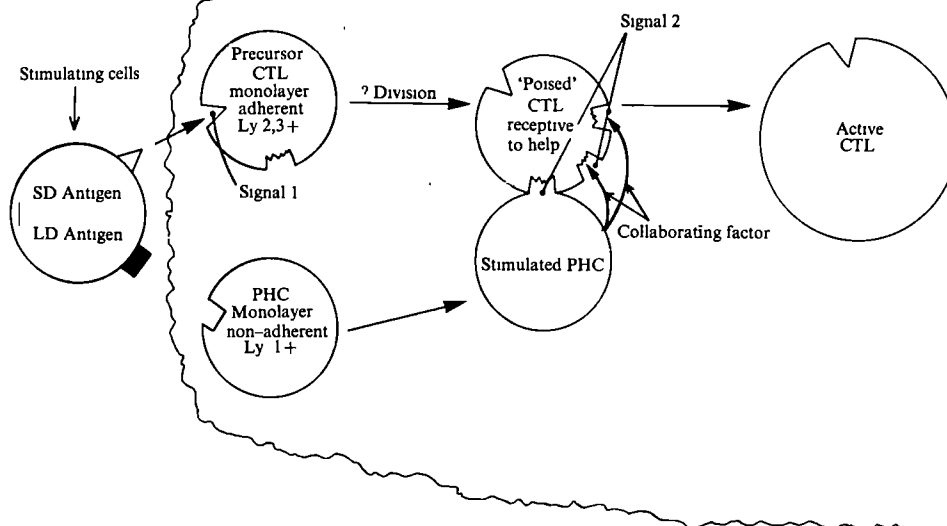
Table 3b Response of B10.A thymocytes to various regions of the H-2 complex

Host strain	Number of hosts	% activity recovered in spleen*	H-2 region difference
AQR	6	0.03 (0.01-0.04)	K
B10.A(4R)	4	0.02 (0.02-0.02)	I-B, I-C, S, G, D
B10	6	0.36 (0.17-0.47)	Complete
B10.A	5	0.02 (0.01-0.03)	None

\*Median (range).

The response of thymocytes to various regions of the H-2 complex is assayed by studying the incorporation of radioactive iodouracil-deoxyriboside (IUdR) into the spleens of recipient animals. The % activity recovered in spleen is in relation to the entire radioactivity injected. Control values usually range between 0.02 and 0.05%; a value of 0.36% is highly significant. (After Elkins *et al.*<sup>50</sup>.)

rejection<sup>54</sup>. Other transfused patients who do not have antibody directed at donor SD antigens more readily accept and retain allografts than non-transfused transplant recipients. van Rood has suggested this increased graft survival after many transfusions results from immunisation to LD determinants and subsequent production of an antibody that enhances graft survival (results presented at the annual meeting of the AASCT, 1975). Such enhancement has been demonstrated in mouse by passive transfer of anti-Ia antisera that prolong graft survival even when administered in  $\mu\text{g kg}^{-1}$  quantities; anti-SD antibody did not enhance graft survival<sup>55</sup>. The apparently differential role of



**Fig. 4** Primary response. A stimulating cell carrying both an SD antigen(s) and an LD antigen(s) will evoke separate responses in two T-cell subpopulations in a manner similar to that depicted in the original cellular model proposed to explain the LD-SD dichotomy<sup>41</sup>. We can refer to the recognition of the SD antigen by the precursor CTL as signal 1. This cell is monolayer adherent and Ly-2,3+. It seems, on the basis of results shown in Table 4, that when the LD and SD stimuli are added at different times after initiation of culture, the SD antigens initiate a step in differentiation which may be independent of LD. This differentiation step results in a "poised" CTL which is receptive to help. A separate cell population of T lymphocytes, the proliferating helper cells, respond to LD antigens. This cell is monolayer non-adherent and Ly-1+. The help given by the PHC to the poised CTL might be delivered either by cell-cell contact between the PHC and the poised CTL or by a "collaborating factor" (secreted from the stimulated PHC) to which the poised CTL can respond. This is an obvious analogy to the model for T-B cell collaboration. It is a purely gratuitous assumption that the receptor on the CTL that recognises the collaborating factor is present in an effectively increased number on the cell as compared with the precursor CTL. Many alternative mechanisms may account for the fact that the poised CTL is more receptive to help than the precursor CTL. The direct contact between the stimulated PHC and the poised CTL or the collaborating factor would provide signal 2 to the CTL and lead to further differentiation to an active CTL. To what degree a CTL receiving only signal 1 can become cytotoxic and the other aspects of this model are discussed in this article. We have recently elaborated on this model to include the "secondary" responses of lymphocytes in culture<sup>58</sup>.

antibody directed at Ia (?LD) and SD molecules once again is consistent with a dichotomy of function.

### An attempt at synthesis

We have discussed above data supporting the contention that LD and SD antigens are functionally distinct and that two separable populations of T lymphocytes respond to these antigens. We shall now attempt to expand on our previous model to explain how LD and SD antigens and their respective responding lymphocyte populations interact in the generation of a specific cytotoxic response. The elaboration is based on the Bretscher-Cohn model for B-cell activation<sup>56</sup> and is in general agreement with Crichton and Lafferty<sup>57</sup>. It would suggest that the CTL receives two signals for activation: "signal one", the SD antigen, and "signal two" given by the PHC after it has been stimulated by LD. It is quite likely that many stimuli will, either through the PHC or by acting directly on the CTL, be able to provide signal two. This model is illustrated in Fig. 4. In this context the LD-SD dichotomy can be summarised as follows. (1) The SD antigens function as the prime CTL activating determinants and as targets for these cells. The weak proliferative response stimulated by SD region differences in mouse may reflect CTL division or stimulation of an SD reactive PHC population. Alternatively the proliferative response is due to PHCs responding to LD determinants included in the "SD region difference". (2) A strong MHC LD locus, separable from the SD loci, is primarily responsible for stimulating the strong proliferative response in MLC and presumably for inducing the PHC-CTL helper effect. One or more weaker MHC LD loci probably exist. Weak but significant cytotoxicity is often associated with I region differences; it is not established

whether this is directed against LD as opposed to other I region antigens. (3) There is very substantial and clear evidence for LD-SD collaboration based on both genetic and cellular studies. The presence of an LD stimulus in the sensitising MLC markedly enhances the development of cytotoxicity aimed at the SD region targets unless maximum CML is already present (M. B. Widmer, F.H.B., H. R. McDonald and J. C. Cerrotini, unpublished) *in vitro* due to the presence of a strong signal 2 such as may be provided by heterologous serum or conditioned medium, even though stimulation by LD alone does not generate cytotoxicity against the SD target. It is not known whether the LD helper effect is essential and the CML generated in SD region different strains is due to an LD-like stimulus determined by gene(s) in that region, or that LD simply influences the quantitative amount of CML induced by SD alone. The suggestion that the SD antigens actually initiate (signal 1) a differentiative response that is expanded, or allowed to differentiate further, by the presence of cells responding to the LD stimulus (signal 2) is based on kinetic experiments in which SD and LD stimuli are added to responding cell populations at different times. The basic findings<sup>58</sup>, which look quite similar to those on T-B cell collaboration, are illustrated in Table 4. If the SD different stimulus (in a "three-cell" protocol) is added at time zero and the LD stimulus is delayed for 24 h, the CML against B10.A target cells is in fact increased, although the kinetics of the development of the cytotoxic response aimed at the SD antigens may differ (see day 5). Adding the 6R<sub>x</sub> cells 48 h after initiation of the AQR(B10.A)<sub>UV</sub> culture still results in 48.7±4.8% CML on B10.A target cells. In the reciprocal direction, delay in adding the SD different cells, after adding the LD different cells on day zero, results in a similar delay in the development of CML. We have

discussed this in detail elsewhere<sup>38</sup>. Most important, the presence of the SD stimulus does not seem to potentiate "anti-LD" CML. This is in contrast to the potentiation of anti-SD CML by an LD stimulus, and further supports the LD-SD dichotomy.

Two findings might seem to contradict the need for signal 2 in the generation of CTLs. First, that Ly-2,3+ lymphocytes alone can respond to an entire MHC difference and become CTLs<sup>49</sup>. This apparent circumvention of the PHC role could be explained by several models. First, a relatively weak signal 2 can be given directly to the CTL by mechanisms other than PHC collaboration either by the SD antigens themselves or some other stimulus. Second, the Ly-2,3+ cells may be a heterogeneous population, a portion of which can respond to the LD antigens and thereby provide the helper effect to allow CTLs within that population to develop. This is consistent with the finding in those same studies that an Ly-2,3+ population depleted of Ly-1+ cells still shows a highly significant proliferative response to strains differing for only the I and S regions (that is, LD different-SD identical strains) without showing CML activity<sup>45</sup>. The lack of cytotoxicity in this situation suggests that the proliferation seen is not due to division of CTLs. Third, the Ly-1+ cells that proliferate in response to LD (proliferating "helper" cells) may not be required for the induction of CTLs but may simply expand an ongoing response and may be functionally distinct from Ly-2,3+ LD reactive cells. Finally, it may be that PHCs in the stimulating cell population respond to LD antigens on the responding cells and provide the helper effect to the responding CTLs. If help is provided by means of a factor, the observation that mitomycin-C treated cells do produce factors is important<sup>50</sup>.

The second apparent contradiction is the finding that an SD region difference alone can stimulate both proliferative and CML responses in mouse. Ultraviolet irradiation of stimulating cells abrogates their ability to evoke a proliferative MLC response thus appearing to ablate LD expression; the SD antigens on ultraviolet-treated cells can still be detected serologically<sup>60</sup>. In Table 4 the SD region different stimulating cells are treated with ultraviolet irradiation rather than mitomycin-C<sup>38</sup>. In these circumstances the cells evoke minimal if any proliferative or cytotoxic response. The addition of an LD stimulus to the sensitising mixture however still leads to a very marked cytotoxic response against the SD different target cells.

The three-cell experiments with ultraviolet-treated stimulating cells show that an SD stimulus that is effective at activating CTLs is not capable of strongly stimulating the PHC and does not by itself lead to as great a cytotoxic reaction as in the presence of LD. At least four mechanisms could be invoked to explain the difference between the mitomycin-C treated (see Table 2) and ultraviolet-treated SD region disparate stimulating cells. First, the same determinant on the SD molecule may be responsible for activating the PHC and CTL but the two cell populations have different requirements for activation (possibly due to molecularly different recognition molecules). Treatment with ultraviolet light leaves a cell still capable of activating CTLs but not PHCs. For example, the cell surface density of SD antigens may be reduced by ultraviolet treatment making the SD stimulus ineffective at stimulating PHCs or providing signal 2 directly to the CTL. Second, cellular contact initiated by recognition of surface SD antigen might allow a cell membrane interaction (possibly unrelated to antigen recognition) to provide a weak substitute for signal 2. Ultraviolet-treated stimulating cells provide the SD stimulus (signal 1) but are unable to provide the substitute signal 2 and therefore require an additional generation of signal 2 provided by the LD stimulus by way of PHCs. Third, an ultraviolet-sensitive part of the SD molecule different from that triggering the CTL may activate the PHC. This is similar to our initial analogy of a carrier-hapten system to the LD-SD problem<sup>15,35</sup> and the reiteration of that model applied to the SD antigen<sup>65</sup>. Fourth, there may be LD-type loci in the K region separable from the SD loci<sup>32</sup>. Conceptually, in terms of phenotypic expression, the third and fourth models both suggest that the LD and SD antigens are determined by genetically separate sites; the question whether the LD and SD stimuli are determined by separate genes or separate subclonogenic segments is not terribly important in this context.

## Mutants of H-2

The studies that first led to the definition of H-2 LD loci<sup>15,16,62</sup> were done using a mouse strain, discovered by Bailey, that carries a spontaneous mutation in *H-2* to the left of *Ss*<sup>63</sup>. The phenotypic expression of the mutation is reciprocal skin graft rejection between the parental strain, C57BL/6, and the mutant, H(2l), in spite of apparent serological identity of cells of the two strains. More recent

Table 4 Interaction kinetics

Effector combination	MLC <sup>3</sup> H-TdR incorporation (c.p.m. $\pm$ s.d.) day 5	Target cell	% CML (mean $\pm$ s.d.)	
			Day 5	Day 6
AQR+6R <sub>x</sub> +B10.A <sub>UV</sub>	25,802 $\pm$ 1,090	AQR	-0.57 $\pm$ 1.5	-5.8 $\pm$ 2.8
		6R	13.6 $\pm$ 2.1	17.5 $\pm$ 5.9
		B10.A	35.9 $\pm$ 3.5	50.0 $\pm$ 4.4
AQR+6R <sub>x</sub>	26,184 $\pm$ 1,057	AQR	-1.7 $\pm$ 1.1	-10.4 $\pm$ 3.2
		6R	15.1 $\pm$ 1.9	18.8 $\pm$ 3.2
		B10.A	-0.6 $\pm$ 2.0	-4.1 $\pm$ 4.7
AQR+B10.A <sub>UV</sub>	1,520 $\pm$ 104	AQR	-3.2 $\pm$ 0.9	-12.5 $\pm$ 2.0
		6R	-2.3 $\pm$ 1.8	-12.3 $\pm$ 2.5
		B10.A	1.5 $\pm$ 1.7	8.1 $\pm$ 2.8
AQR+B10.A <sub>UV</sub> +6R <sub>x</sub> (24 h)	27,863 $\pm$ 462	AQR	-0.5 $\pm$ 0.8	-7.5 $\pm$ 2.0
		6R	-0.6 $\pm$ 2.2	11.2 $\pm$ 3.0
		B10.A	5.2 $\pm$ 1.7	70.0 $\pm$ 7.9
AQR+6R <sub>x</sub> +B10.A <sub>UV</sub> (24 h)	38,224 $\pm$ 1,339	AQR	-0.5 $\pm$ 0.5	-3.5 $\pm$ 2.0
		6R	13.9 $\pm$ 1.8	14.9 $\pm$ 4.2
		B10.A	12.1 $\pm$ 1.6	40.3 $\pm$ 7.1
AQR+AQR <sub>x</sub>	975 $\pm$ 34	AQR	-2.1 $\pm$ 0.9	-11.1 $\pm$ 2.7
		6R	-1.5 $\pm$ 1.7	-12.2 $\pm$ 3.6
		B10.A	—	8.3 $\pm$ 3.0

The control effector combination, AQR+AQR<sub>x</sub>, gives 8.3  $\pm$  3% CML on the B10.A target cells. It is thus difficult to know whether there is any significant killing on B10.A target cells when AQR cells are stimulated with B10.A<sub>UV</sub>. A 24-h delay in adding the B10.A<sub>UV</sub> cells results in a level of cytotoxicity on B10.A target cells on day 6 not significantly different from that found on day 5 when the LD and SD stimuli are added together at time zero. (D. J. Schendel and F. H. B., *Eur. J. Immun.*, in the press.)

studies suggest that a serological difference may exist<sup>64,65</sup>. Consistent with this possibility are recent findings that the SD antigenic products of BL/6 and H(zl) differ by peptide mapping (J. L. Brown and S. Nathanson, personal communication). In MLC and CML, both a proliferative response and a very marked cytotoxic reaction were seen reciprocally (see Table 5); the magnitude of the cytotoxic response between cells of the parental and mutant strain is comparable to that observed in the responses between mouse strains known to differ for both LD and SD antigens, and significantly greater than that observed between strains differing only for an SD region. The existence of both the MLC proliferative response and CML cytotoxicity related to these mutants has been extensively discussed<sup>24,62,64,65</sup>.

It is possible that the spontaneous mutants carry "point mutations" that affect only a single phenotypic product of the MHC, the SD molecule. By this assumption one must explain why CML equal to that found in LD+SD different strains is present in the C57BL/6-H(zl) combination. Two considerations may be helpful. The basis on which these mutants were found, that is relatively rapid skin graft rejection, may have selected for highly immunogenic SD antigens that can alone account for these findings. In addition, proliferative responses between C57BL/6 and the mutants are on the average weaker than those seen in H-2 disparate strains. This would again be consistent with a highly immunogenic SD difference that by itself induces a sufficient helper proliferative response (or in some other way gives signal 2) to allow strong CML to develop. We do not know how great the helper response must be for CML to develop, and a relatively low response may in this case be enough. The discussion above of the apparent circumvention of LD-SD collaboration in SD region different strains alone is also applicable in this instance.

The spontaneous derivation of H(zl) does not prove that the mutant differs only for an amino acid substitution in the SD gene product. Subsequent skin graft screening of C57BL/6 animals revealed a surprisingly large proportion (more than 1%) of independently derived H-2 mutants<sup>78</sup> suggesting a somewhat non-conventional mutation mechanism. Because F<sub>1</sub> hybrids of any two of these H-2 mutants reject parental skin grafts, it seems that at least one common genetic determinant was altered in each independent mutational event; however, these "complementation" studies do not exclude the possibility that the mutational event also resulted in changes of other MHC controlled determinants. It does not seem unreasonable to hypothesise that a spontaneous mutation would affect the phenotypic expression of more than a single molecule. Several mechanisms could be suggested.

First, it may be that spontaneous mutation affects only one molecule in terms of an amino acid substitution, for instance the SD antigen. That SD antigenic molecule may interact in a physically intimate manner with the LD antigen so that a change in the SD antigenic molecule may result in a change in the expression of the LD antigen. We have recently discussed possible LD-SD epistasis in MLC reactions<sup>66</sup>. Arguing against this possibility is the lack of any such finding in mouse strains carrying recombinant chromosomes. Second, it may be that these mutant strains were generated by the effects of complex mutation events. By this model we predict that the LD antigens, of these mutant strains, when isolated, will have one (or more) amino acid substitution(s) from the parental LD, as does the SD molecule in the case of H(zl). Third, it may be that mutations of LD and/or SD products are not infrequent and are, in fact, carried by many inbred lines. Let us hypothesise that rapid skin graft rejection between the two strains is dependent on differences for both LD and SD-type antigens. The mutants mentioned here were identified by skin grafting only; the parental mice were not routinely screened for mutations in LD alone, as detected in MLC,

Table 5 MLC and CML studies in the H-2 mutant H(zl)

a, MLC data		
Responding cell	Stimulating cell	<sup>3</sup> H-TdR incorporation (c.p.m.)
B6*	B6 <sub>x</sub>	3,409 ± 962
B6	H(zl) <sub>x</sub>	36,665 ± 5,310
B6	7R <sub>x</sub> *	40,620 ± 1,507
H(zl)	H(zl) <sub>x</sub>	2,001 ± 974
H(zl)	B6 <sub>x</sub>	24,677 ± 7,697
H(zl)	7R <sub>x</sub>	68,727 ± 14,468
b, CML data		
Responding cell (effector)	Stimulating (sensitising) cell	Target cells
		B6                      H(zl)                      S*
B6	H(zl) <sub>x</sub>	5.89 ± 5.01†      89.43 ± 5.15      9.79 ± 3.00
B6	S <sub>x</sub>	-0.15 ± 2.05      23.72 ± 9.08      55.95 ± 6.94
H(zl)	B6 <sub>x</sub>	57.45 ± 14.13      -0.26 ± 4.13      0.76 ± 1.92

\*B6 = C57BL/6By; S = B10.S; 7R = B10.S(7R).

†% CML ± s.d.

(From M. Widmer, unpublished.)

or for mutations in SD alone, as detected in "three-cell" CML protocols. It is thus possible that some of the parental BL/6 lines may have been carrying either an LD or an SD mutation that was not detected by skin grafting. Subsequent mutation could then yield "spontaneous" mutants identified by skin grafting because they now differed from the original parental line for both LD and SD antigens.

Any one of these explanations as well as other models would explain the complex phenotypic expression in these mutants; the biochemical isolation and characterisation of LD and SD phenotypic products will be of tremendous benefit in understanding the LD-SD dichotomy, particularly with respect to these mutants. Clearly, the LD-SD model is an oversimplification of the genetics of histocompatibility antigens within the MHC; given our present state of knowledge, the model of a functional dichotomy of LD and SD antigens is consistent with all data collected so far, including these mutants, and serves as an heuristically valuable model.

## Questions of genotypic identity

Responses of T lymphocyte subpopulations have been ascribed to antigens associated with a given H-2 or HLA region. In considering the genetic relationship of these antigens and the other functions ascribed to the same MHC (sub)regions, three basic questions of identity arise: (1) are the target antigens recognised by the CTLs identical to the SD molecules or, even more specifically, the determinants on the SD molecules that are recognised by antibody? (2) Are the LD antigens identical to the Ia antigens? (3) Are the LD antigens identical to the products of the I region locus<sup>48,61</sup> causing skin graft rejection?

We and others have in the past discussed whether one can equate SD antigens with CTL target determinants<sup>24,62,67,68</sup>. Data presented in Table 6 demonstrate that human CTLs sensitised to lyse cells from the stimulating cell donor can also lyse target cells from a third party not sharing any serologically detectable cross reactivities with the stimulating cell donor. Experiments using a CML "blocking" technique have demonstrated that this cross killing is specific<sup>68</sup>, cytotoxicity of target cells from a third party results from CTL recognition of shared antigens on the initial stimulating cells and the target cells. Family studies have demonstrated<sup>69</sup> that these serologically undetected shared antigens are genetically controlled within the MHC.



Table 6 CML cross killing

CTLs	W	Target X	Y	Z
ZW <sub>m</sub>	62.5±2.8 (25)	55.9±3.2 (10)	38.5±2.7 (2)	1.7±3.9
ZX <sub>m</sub>	48.4±4.3 <sup>a</sup> (10) <sup>b</sup>	67.6±6.2 (25)	29.3±3.5 (1)	0.3±3.1
ZY <sub>m</sub>	40.7±3.8 (6)	28.6±2.3 (2)	58.9±3.4 (25)	-1.6±3.2
Z--	0.4±1.7	-3.1±2.3	-1.7±2.5	—

CTLs from the indicated mixed leukocyte cultures as well as unstimulated cells from Z, were tested on target cells from individuals W, X, Y and Z. No cross reacting or shared antigens were detected serologically among individuals W, X and Y. Typing results are as follows: W = (1, 8); X = (2, 12); Y = (10, 7, 27); Z = (10, 11, W15, W16). (In other experiments similar results were obtained with all four antigens of HLA-A and HLA-B defined in every individual.) Percentage cytotoxicity by  $25 \times 10^4$  CTLs on  $1 \times 10^4$  target cells are shown. Potency values in parentheses represent the number of specifically sensitised cells from Z that cause the same percentage cytotoxicity as  $25 \times 10^6$  of the CTLs being examined. Potency values were interpolated from a log-linear graph of several specific CTL concentrations against percentage cytotoxicity. Control values for each target (<sup>51</sup>Cr c.p.m.): W, S.R. =  $211 \pm 20$ , Max. =  $1,913 \pm 22$ ; X, S.R. =  $206 \pm 16$ ; Max. =  $1,089 \pm 46$ ; Y, S.R. =  $246 \pm 30$ ; Max. =  $1,597 \pm 50$ ; Z, S.R. =  $322 \pm 42$ , Max. =  $1,714 \pm 72$ . (From ref. 68.)

These data could thus be interpreted to suggest that the target for the CTLs is determined by MHC genes that are different from those determining the SD antigens, a suggestion made in our initial study with the C57BL/6-H(zl) mutant combination<sup>62</sup>. Alternatively, the cytotoxic target antigen may be the SD molecule itself, that is the same molecular determinant that evokes antibody production is recognised by CTLs; the antibody-producing B cell and the CTL, however, recognise the same determinant with different specificity, possibly by molecularly distinct receptor molecules, as suggested recently<sup>67</sup>. It is also possible that the CTLs recognise a different portion of the SD molecule from that recognised by B-cell produced antibody. The recent findings in mouse that CTLs may react with not only the private but also the public antigens of H-2 SD molecules may provide an explanation<sup>70</sup>; in man it is unlikely that antisera detecting public HLA SD antigens are frequently used. There is direct evidence that the CML targets are not the SD molecules<sup>71,72</sup>.

Evidence suggesting at least partial identity of Ia and LD determinants<sup>73</sup> demonstrated that anti-Ia antisera blocked the stimulating determinants in an MLC<sup>74</sup>. These findings can be used to argue that LD and Ia are, at least in part, identical; the ability of many different antibodies to block MLC reactions, however, prevents the firm acceptance of this last conclusion. In human studies, it has been noted by van Rood and his colleagues that there seem to be exceptions to the reaction patterns of the anti-human Ia antisera and the presumed LD determinants carried by those cells<sup>75</sup>. Even if LD antigens can be so defined, we would stress the difficulties of equating Ia antigens (detected by antibody) with LD antigens (detected by T cell reactivity in MLC). This caution is emphasised by the results we have discussed above in considering whether the antigen recognised by CTLs is the same SD antigen detected by alloantisera. In fact, there is no critical evidence that LD "determinants" are expressed on the cell surface.

A locus in the I region leads to skin graft rejection<sup>48,61</sup>. It might be reasonably argued that this locus is identical with the LD locus. One would then have to explain why LD-like differences in other parts of the H-2 complex are not associated with skin graft rejection. At least two possible explanations exist. It may be that I-A sub-region LD antigens collaborate with other I-A determined antigens (perhaps Ia) that are the targets for the CTLs; no such targets may exist in association with LD antigens in other regions. Alternatively, the LD antigens are the targets for the rejection response; the reason why skin graft rejection

may only be associated with LD differences in the I-A subregion is because these are the strongest LD differences of H-2. A threshold level of incompatibility may be needed before skin graft rejection will proceed.

## Summary

We have emphasised the functional dichotomy of MHC LD and SD antigens as well as the differences in cellular responses to these antigens. Perhaps in so doing we have failed to stress adequately the similarities that exist. But while the similarities (for example skin graft rejection associated with both K and I region differences) are so very clear, the differences have best allowed our progressive understanding of MHC induced cellular responses from the perspective stressed in this article. Of greatest importance to our understanding of these transplantation antigens are the potentially differential roles for the LD and SD antigens in the complex series of events that are collectively referred to as the "allograft reaction". It has been suggested that these differences may be "merely quantitative"<sup>61</sup>. This possibility has been discussed repeatedly in our previous reports on the distinction of LD and SD. In fact, the great bulk of biological phenomena can be reduced to quantitative differences. It would seem to us that sufficient evidence for such differential activity exists to make the LD-SD dichotomy model an heuristically valuable one for purposes of designing future experiments. We have discussed the clinical relevance of this model elsewhere<sup>23</sup>.

Many authors have speculated<sup>41,76,77</sup>, and evidence has been gathered<sup>19,20</sup> to suggest, that cell surface antigens associated with the MHC are important in developmental and other cell interactions. Some studies have directly addressed the question of the need for MHC compatibility to allow cell interaction to proceed optimally. It thus seems most appropriate that the genetic complex with which we are dealing has been termed the major histocompatibility complex; allowing for the literal interpretation of this term this may be the genetic region that by its influence on "tissue compatibility" may control critical cellular interactions in addition to those observed in allograft reactions. It is the simple good fortune for those whose attention was focused on this complex by transplantation problems to find themselves with a panorama of biological phenomena that require extensive experimental probing and integration, hopefully ultimately leading to an understanding of the MHC in a broader context than has to date been possible.

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## articles

# Two origins of replication in composite R plasmid DNA

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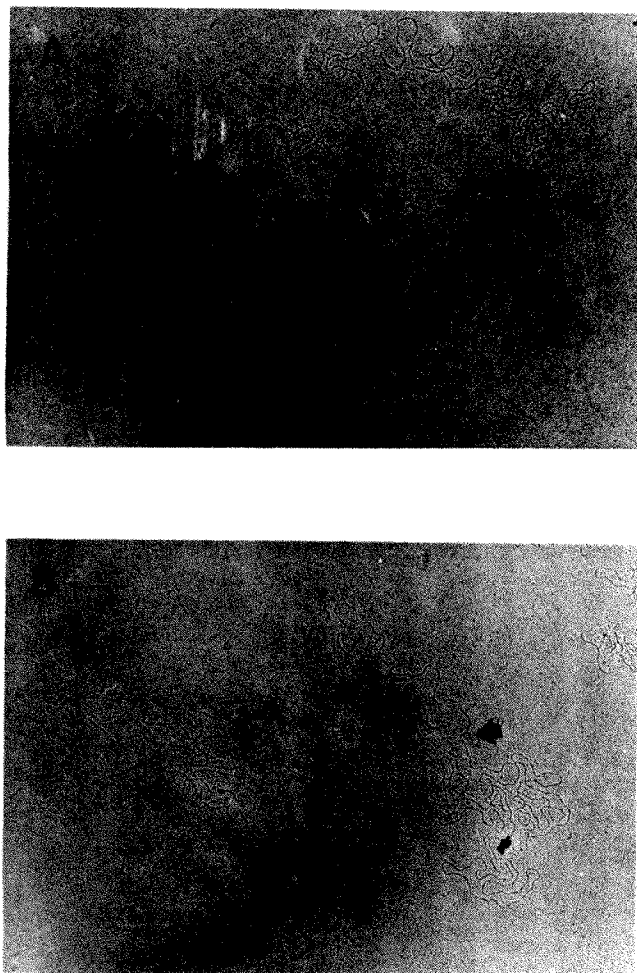
*Electron microscopic examination of a composite R plasmid made up of an RTF component and an r-determinants component shows that replication may originate from either the RTF origin or the r-determinants origin of replication.*

MANY of the drug resistance plasmids (R plasmids) of the Enterobacteriaceae are composite structures consisting of two distinguishable components: a resistance transfer factor (RTF) which mediates the transfer of these plasmids during bacterial mating and an r-determinants component which harbours most of the drug resistance genes of the plasmid<sup>1-3</sup>. Each of these components is capable of autonomous replication in certain host strains of bacteria; that is, each is a "replicon"<sup>4-6</sup>. In most genera of the Enterobacteriaceae the RTF and r-determinants components are united to form the composite R plasmid structure<sup>1,2,4,6-8</sup>. This is also true in *Proteus mirabilis* when this host strain is cultured in drug-free medium<sup>7,9,10</sup>. When *P. mirabilis* is cultured in medium containing any of the drugs to which the r-determinants confer resistance, R plasmids are formed

which consist of a single copy of the RTF and multiple, tandem copies of the r-determinants component (poly-r-determinant R plasmids)<sup>2,5-7,9,10</sup>. Autonomous poly-r-determinants consisting of multiple copies of the r-determinants component are also formed<sup>9</sup>. Since both the RTF and the r-determinants component must contain an origin at which replication is initiated, the replication of R plasmids in *P. mirabilis* provides a model system for studying the replication of chromosomes containing multiple origins of replication. Here we show that either of the two origins of a composite R plasmid can be used for the initiation of R plasmid replication.

We have previously shown that there is a substantial increase in the fraction of replicating R plasmid DNA in *P. mirabilis* cells as a consequence of decreasing the rate of DNA chain elongation by limiting the concentration of DNA precursors<sup>10,11</sup>. Using this technique, three experiments were carried out to isolate replicating molecules of R plasmid NR1 DNA from *P. mirabilis*. In the cell culture conditions used, the R plasmid DNA exists as the undissociated composite structure (circular contour length of 37  $\mu$ m; molecular weight  $63 \times 10^6$ ) which consists of a single copy of the RTF (29  $\mu$ m; molecular weight  $49 \times 10^6$ ) and a

single copy of the r-determinants component ( $8.3 \mu\text{m}$ ; molecular weight  $14 \times 10^6$ )<sup>7,9-11</sup>. In two of the experiments thymine limitation was used to increase the fraction of replicating R plasmid DNA; in the third experiment treatment of the cells with hydroxyurea was used<sup>11</sup>. Fractionated R plasmid DNA was spread for electron microscopy at pH 10.7 so that approximately 20 small denaturation sites



**Fig. 1** Two examples of replicating molecules of composite R plasmid NR1 DNA. The two arrows indicate the two branch points (replication forks) of the replicating molecules. These two replicating molecules were isolated from R<sup>+</sup> *P. mirabilis* cells after addition of hydroxyurea to the growth medium. One molecule has initiated replication in the RTF component (A) and the other in the r-determinants component (B) of the composite R plasmid DNA. These molecules are designated as H-A and H-B in Fig. 2.

would be present in the R plasmid DNA<sup>12</sup>. We could thus compare the distribution of denaturation sites in the replicating molecules with the denaturation map of composite NR1 DNA. This made possible the orientation of the replicating molecules in a unique manner. The orientation of the replicating molecules was greatly facilitated by locating the  $8\text{-}\mu\text{m}$  r-determinants region which has very few denaturation sites when the DNA is spread for electron microscopy at pH 10.7 (ref. 12).

The replicating NR1 DNA molecules observed in the electron microscope were double-branched ( $\theta$ -type) circular structures when, as will be discussed subsequently, either the RTF origin (Fig. 1A) or the r-determinants origin (Fig. 1B) was used for the initiation of replication. The replicated regions (daughter branches) of the molecules were found to have equal contour lengths. Although

approximately 5% of the molecules observed in the electron microscope contained branch points (replication forks), only about one-fourth of these was suitable for detailed analysis. The criteria for choosing replicating molecules for mapping were as follows: (1) at least 10, and as many as 20, denaturation sites in each molecule were preferred for the most accurate alignment of the molecule with the NR1 DNA denaturation map; (2) the locations of the two replication branch points were clear and unambiguous; (3) there was no ambiguity in following the contour of the molecules throughout their entire length.

In Fig. 2 the distribution of denaturation sites and the replication branch positions in aligned molecules of replicating R plasmid DNA are superimposed on the composite NR1 DNA denaturation map. For convenience of presentation, the circular NR1 DNA molecules are represented by a linear denaturation map. The r-determinants component is located to the left side of the map (0.0–0.23) and the RTF component to the right side (0.23–1.0)<sup>12</sup>. Replicating molecules from the two thymine limitation experiments and the hydroxyurea experiment are designated T1, T2, and H, respectively. The weight-average histogram of the distribution of denaturation sites in all of the replicating molecules shown in Fig. 2 is presented in Fig. 3a. This histogram agrees well with the weight-average histogram (denaturation map) previously determined for non-replicating NR1 DNA molecules<sup>12</sup> (Fig. 3b).

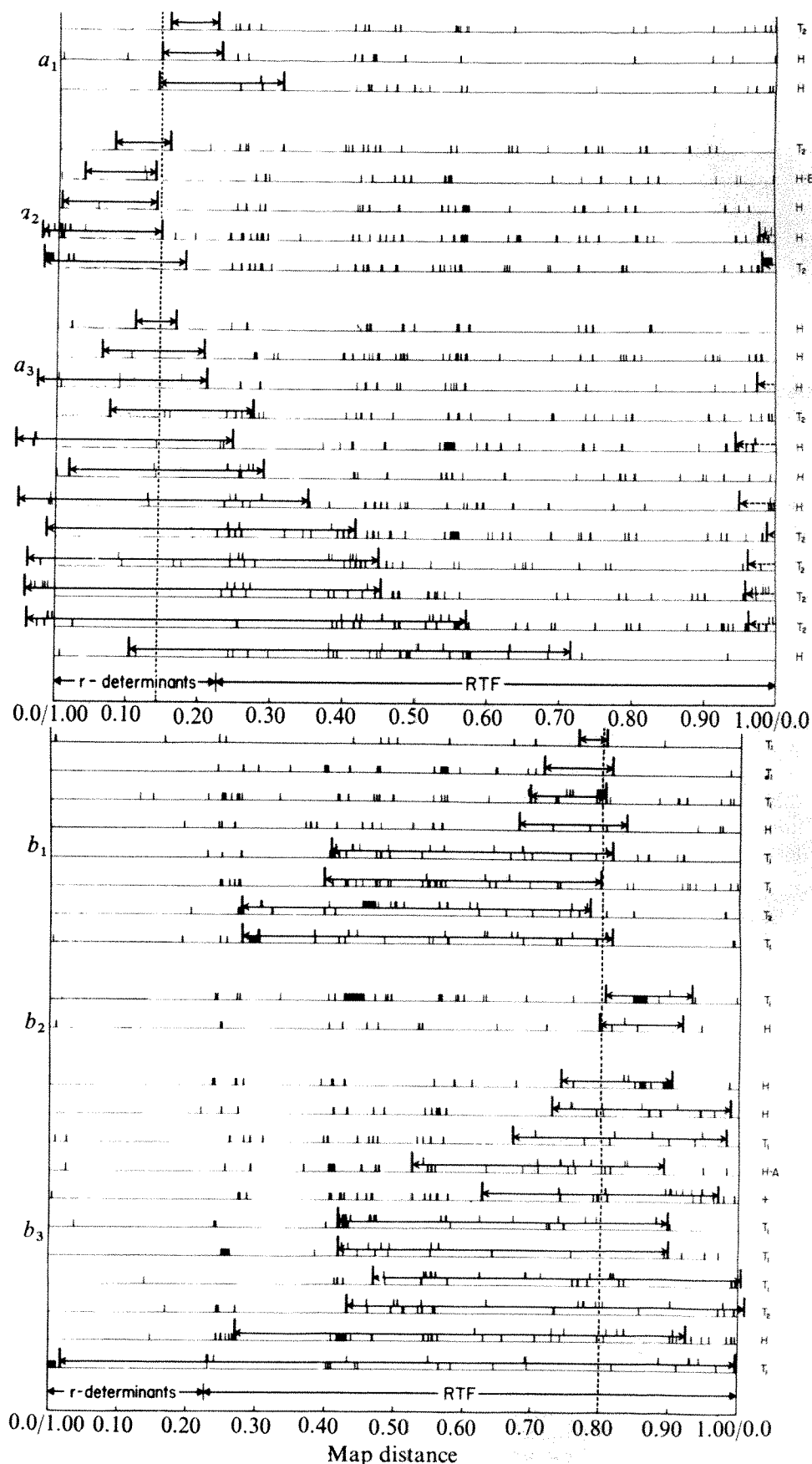
The extent of replication and the branch positions of replicating R plasmid molecules are designated by arrows in Fig. 2. The positions of the replication branches are consistent with two different origins at which replication of the composite R plasmid DNA can be initiated. One is located in the r-determinants component at a position of 0.14 on the denaturation map. The other is located in the RTF component at a position of 0.80 on the denaturation map. The replicating molecules with the shortest replication branches were consistent with only these two origins and helped to localise their positions on the map.

Replicating molecules isolated from the same culture of cells were found to have initiated replication at either of these two origins of replication. In the case of either origin, replication could be either bidirectional or unidirectional in either sense. The replication branch points were usually not symmetrically arranged about either origin of replication. Replicating molecules have not been observed in which both origins were used simultaneously (that is, with four branch points). Such molecules may, however, be much more likely to fragment during isolation and handling of the DNA which would diminish their proportion in the population of replicating molecules.

A single replicating molecule has been observed by chance in an exponential culture which had not been subjected to substrate limitation. The replication of this molecule (designated as + in Fig. 2b) was initiated at the RTF origin and replication was bidirectional.

In many replicating molecules the branch point to the left of the r-determinants origin and the branch point to the right of the RTF origin tend to cluster around the position 0.95–1.00 on the denaturation map, suggesting that this position could be a terminus of replication. For several reasons it seems unlikely that this position is a third origin of replication. First, the positions of the two branch points of all of the replicating molecules which were analysed are consistent with either the r-determinants origin or the RTF origin. Second, none of the molecules with a small extent of replication (less than 15%) was consistent with an origin at 0.95–1.00. The existence of a terminus of replication at 0.95–1.00 could explain the asymmetry in the distribution of the replication branch points about the two origins since the arrival of one of the replication forks at the terminus would interrupt replication in that direction.

**Fig. 2** Denaturation sites and replication branch positions in aligned molecules of replicating R plasmid NR1 DNA superimposed on the composite NR1 DNA denaturation map. A thymine auxotroph of *P. mirabilis* harbouring the composite R plasmid NR1 was cultured in drug-free M9 minimal medium containing 0.06% casamino acids and other supplements<sup>11</sup>. In two of the experiments (T1 and T2) exponential phase cells were filtered, washed, and resuspended in similar medium containing a limiting concentration of thymine ( $0.05 \mu\text{g ml}^{-1}$ ). In a third experiment (H) a final concentration of 0.075 M hydroxyurea was added to the culture medium. Both substrate limitation conditions reduce the rate of DNA synthesis about 20-fold. After about a 50% increase in cell mass during substrate limitation, DNA was prepared from the cells. Replicating R plasmid DNA molecules were fractionated by caesium chloride preparative density gradient centrifugation<sup>11</sup>. Fractionated R plasmid DNA was spread for electron microscopy at pH 10.7. Replicating, circular NR1 DNA molecules which had on the order of 20 small denaturation sites, were photographed and analysed<sup>12</sup>. The denaturation sites are represented by the smaller vertical bars of varying width, the width of the bars being proportional to the size of the denaturation site. From the distribution of denaturation sites on the replicating molecules, the molecules were aligned relative to the composite NR1 DNA denaturation map<sup>12</sup>. All the replicating molecules shown here are circular structures which have been superimposed on a linear composite NR1 DNA denaturation map for convenience of presentation. The extent of replication and the replication branch positions of individual molecules are designated by the arrows between the two larger vertical bars. Since the replicating molecules are circular, the arrows representing the replication branch positions have been extended through the 0.0/1.00 junction of the denaturation map to avoid interruption. In these cases, the extended part of the arrows is repeated as a dashed arrow at the corresponding region at the other end of the denaturation map. Molecules whose replication branches are consistent with the r-determinants origin of replication are shown in the group of molecules included in *a* and molecules consistent with the RTF origin are shown in the group of molecules included in *b*. The molecules in each of the classes *a* and *b* are divided into three subclasses depending on whether replication is unidirectional to the right (*a*<sub>1</sub> and *b*<sub>2</sub>) or unidirectional to the left (*a*<sub>2</sub> and *b*<sub>1</sub>); replicating molecules showing bidirectional replication are included in *a*<sub>3</sub> and *b*<sub>3</sub>.





All 10 replicating molecules in the first thymine limitation experiment (T1 molecules) were found to initiate replication at the RTF origin. In the case of the T2 replicating molecules and the H replicating molecules, however, about three-quarters of the molecules used the r-determinants origin and only a quarter the RTF origin. Since only a relatively small number of molecules were examined in each experiment, the data do not give a statistically accurate value for the frequency of use of

in eukaryotic chromosomes using electron microscopy<sup>20</sup> and autoradiography<sup>21</sup>, indicating that there are multiple origins of replication. Poly-r-determinant R plasmids<sup>5-7,9,10</sup> must also contain multiple origins of replication, the number depending on the number of copies of r-determinants. Analysis of replicating poly-r-determinant R plasmid DNA by denaturation mapping should make it possible to determine whether there is any specificity involved in the selection of an origin for the initiation of

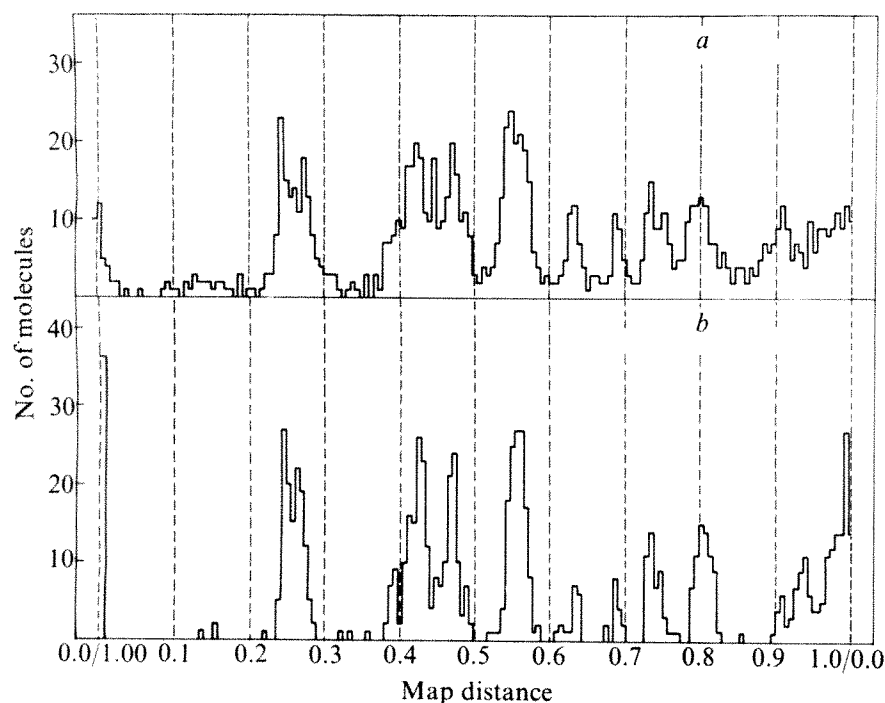


Fig. 3 Weight-average histogram of the distribution of denaturation sites in aligned replicating molecules of composite R plasmid NR1 DNA. *a*, Histogram constructed from the distribution of denaturation sites in the replicating NR1 DNA molecules shown in Fig. 2. The histogram includes all the denaturations present in the replicating molecules, even those present in the sister replication branches of the same molecule. *b*, Denaturation map of non-replicating composite R plasmid NR1 DNA<sup>12</sup>.

either origin for the initiation of replication. Other experiments in which the total replicating plasmid DNA has been analysed (rather than individual replicating molecules in an electron microscope) also indicate that there is preferential use of the r-determinants origin. These experiments involve DNA-DNA hybridisation and buoyant density analysis of replicating R plasmid DNA and Agarose gel analysis of the fragments of replicating R plasmid DNA produced by treatment with restriction endonuclease *EcoRI* (D.P. and R.R., in preparation).

Our experiments have shown that there are at least two functional origins of replication in composite R plasmid NR1 DNA. The use of both origins is of course not mandatory since one origin might be dominant to the other in the initiation of replication. For example, the bacterial chromosome origin is dominant to the F factor origin in an Hfr strain<sup>13</sup> or to a prophage origin in a lysogenic strain<sup>14</sup> of bacteria. It is presently not known what determines the selection of either of the two origins for the initiation of composite R plasmid replication. It is possible that the substrate limitation technique itself could affect the selection. Since R plasmid NR1 replication is arrested when protein synthesis is inhibited<sup>6,15</sup>, it will be of interest to determine whether there are specific R plasmid initiator proteins for each of the two origins or whether the same initiator proteins can function at either of the two origins.

Like phage  $\lambda$  DNA<sup>16</sup>, R plasmid DNA is replicated either bidirectionally or unidirectionally in either sense. Colicin E1 plasmid DNA, on the other hand, replicates unidirectionally from a unique origin<sup>17-19</sup>. Thus, in the two examples of plasmid replication which have been examined, different modes of replication have been observed.

Several internal replicating regions have been observed

replication, whether the additional r-determinants origins on poly-r-determinant R plasmids will influence the frequency of selection of the r-determinants origin relative to the RTF origin, and whether multiple initiations can occur on the same replicating R plasmid DNA molecule.

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# Replication control in a composite plasmid constructed by *in vitro* linkage of two distinct replicons

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*Although it carries two competent replication systems, a composite plasmid formed in vitro by linkage of the complete ColE1 and pSC101 plasmid replicons at their unique EcoRI endonuclease cleavage sites normally uses only the replication origin and functions of the ColE1 component. Restriction of ColE1 replication functions by DNA polymerase I deprivation results, however, in exclusive use of the pSC101 replication origin. When using the ColE1 replication system the composite plasmid is nevertheless incompatible with both the parent replicons. This suggests that a trans-dominant gene product is involved in plasmid incompatibility and supports negative control rather than positive control models for regulation of the initiation of DNA replication.*

INITIATION of DNA replication usually occurs at a unique site on the DNA molecule—the replication origin—by covalent attachment of deoxyribonucleotides to a short chain of primer RNA<sup>1</sup>, designated origin RNA<sup>2</sup>. A few replicons, however, have been reported to contain and sometimes utilise more than one replication origin (*Drosophila melanogaster* chromosomal DNA<sup>3</sup>; mitochondrial DNA oligomers<sup>4</sup>; *Escherichia coli* plasmids NR1 (ref. 5) and RSF1040 (ref. 6)), although little is known about the control of origin usage in such systems.

To gain information on how multiple regions of replication activity (replication origin plus associated essential replication functions) present on a single DNA molecule function and interact, we have constructed a model replicon (the pSC134 plasmid) by *in vitro* linkage of the colicin plasmid ColE1 and the tetracycline resistance plasmid pSC101 from *E. coli* at their unique *EcoRI* restriction endonuclease cleavage sites<sup>7</sup>. Since the ColE1 plasmid cannot replicate in bacteria deficient in DNA polymerase I<sup>8</sup> and the pSC101 plasmid cannot replicate in bacteria not actively engaged in protein synthesis<sup>9</sup>, the two components of the pSC134 composite plasmid have functionally distinguishable replication properties. It was possible therefore to show that each component replication system of the hybrid could accomplish replication of the entire molecule. The present study is an investigation of the functioning of the two replication origins of the pSC134 composite plasmid and its incompatibility properties.

## Location of replication origin of pSC101

Treatment of the composite plasmid pSC134 with *EcoRI* restriction endonuclease generates unit length linear forms of the parent plasmids, ColE1 and pSC101, which can be distinguished easily in the electron microscope by their different sizes (4.2 and 5.8 megadaltons respectively<sup>9,10</sup>). Although it is largely possible to distinguish replication origin usage by the two components of pSC134 by determining the size of the *EcoRI* endonuclease-generated fragments which contain replication 'eyes', the unambiguous identification of such fragments

depends on the determination of origin location, since preparations of *EcoRI*-cleaved DNA may contain some fragments produced by random breakage. The replication origin of plasmid ColE1 has previously been found to lie 18% of the molecule length from the unique *EcoRI* cleavage site of this plasmid. Replication proceeds unidirectionally from this origin<sup>11-13</sup>. The location of the replication origin of the pSC101 plasmid has not been reported, however, and to identify unequivocally pSC101 origin usage by the pSC134 hybrid, we first determined the location of the replication origin of the pSC101 plasmid in relation to the unique *EcoRI* cleavage site of the plasmid.

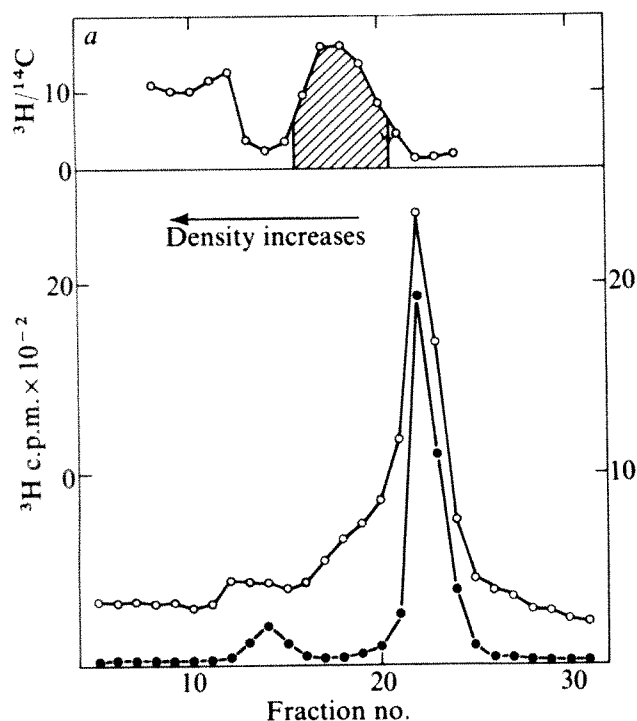
Replicating molecules of pSC101 were prepared by standard methods<sup>6,11</sup> from radioactive thymidine pulse-labelled cells of *E. coli* K12 (pSC101) (Fig. 1a) and examined by electron microscopy after X-ray treatment to relax supertwisted DNA or after *EcoRI* endonuclease digestion (Fig. 1b). Circular replicating molecules were observed to contain a single replication eye, which suggested that the pSC101 plasmid probably contains a single replication origin. This interpretation was confirmed by an examination of *EcoRI* endonuclease-cleaved replicating molecules. Linear molecules which contained a replication eye were photographed (Fig. 1b) and the unreplicated arms and newly replicated segments were measured (Fig. 1c). It may be seen that for molecules less than 50% replicated, one unreplicated arm remains constant in size, whereas the other decreases progressively in length. The pSC101 plasmid therefore replicates unidirectionally. Extrapolation of the plots of relative lengths of unreplicated arms as a function of percentage replication to 0% replication indicates that the pSC101 origin is located about 50% of the unit length from the *EcoRI* cleavage site.

## Replication origin usage by pSC134

To determine whether one or both of the potential replication origins of the pSC134 composite plasmid are active during normal replication of the pSC134 plasmid (that is, in conditions in which neither set of replication functions is deliberately inhibited) and to measure the relative activity of each of the possible origins, replicating molecules of pSC134 were prepared and examined as described for the pSC101 plasmid. Of 97 circular replicating molecules observed (see Fig. 2a for example), only one seemed to have two replication eyes (Fig. 2a, 6). Thus, utilisation of both origins during replication of a single molecule is very rare. Indeed such an event may be an aberrant replication process which would fail to produce functional progeny molecules.

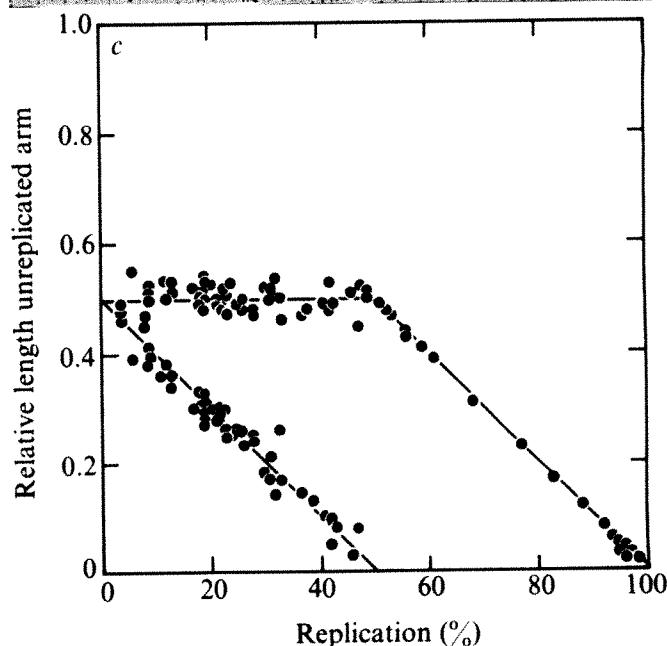
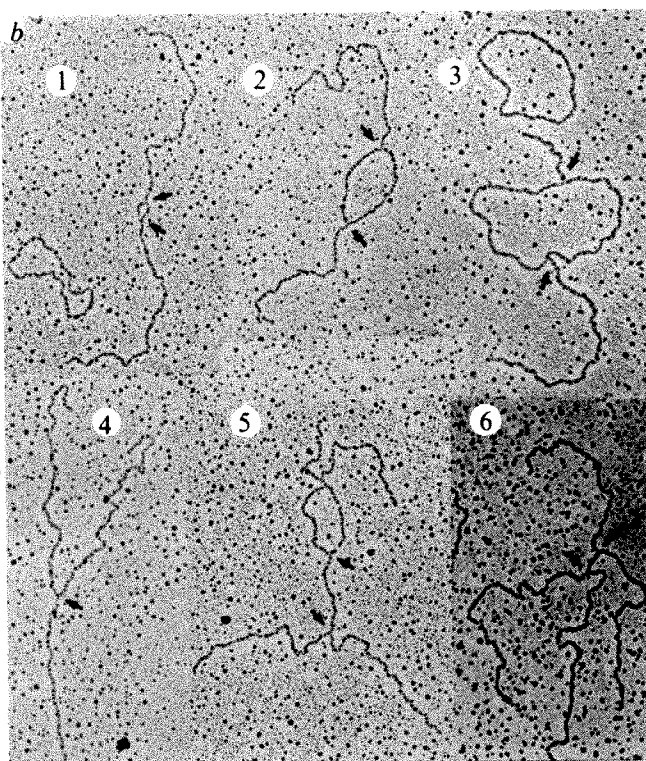
Examination of *EcoRI* cleaved replicating forms of pSC134 revealed that the ColE1 replication origin was used exclusively during normal replication of the composite plasmid: all of 36 *EcoRI*-generated fragments of pSC134 which contained a replication eye had molecular lengths similar to that of ColE1 (Table 1). Furthermore, a plot of the relative lengths of the unreplicated arm against the extent of replication of these fragments (Fig. 2a) indicated that replication of the composite plasmid begins 18% from the nearest *EcoRI*-generated terminus of the ColE1 segment and proceeds unidirectionally away from

that terminus, as reported previously for ColE1 (refs 11–13). Thus, the location of the replication origin and the direction of replication from this origin in the pSC134 composite plasmid are identical to those of the ColE1 parent.



**Fig. 1** Location of replication origin of plasmid pSC101. *a*, Isolation of replicative intermediates. Cells of *E. coli* K12 CR34 (ref. 34) *nal<sup>r</sup>* (pSC101) were grown with aeration at 37 °C in 250 ml M9 minimal medium containing 0.5% casamino acids, 10  $\mu\text{g ml}^{-1}$  thiamine, 3  $\mu\text{g ml}^{-1}$  thymidine and 0.4  $\mu\text{Ci ml}^{-1}$   $^{14}\text{C}$ -thymidine to an  $A_{590}$  of 0.4, centrifuged at 8,000*g* for 5 min at 20 °C, and resuspended in fresh medium without thymidine. The culture was starved for thymidine by incubation at 37 °C for 30 min. It was then cooled to 20 °C and pulse-labelled by addition of thymidine to 0.3  $\mu\text{g ml}^{-1}$  and  $^3\text{H}$ -thymidine to 10  $\mu\text{Ci ml}^{-1}$ . The pulse was terminated after 30 s by pouring the culture into a large flask which was cooled in a dry ice-isopropanol bath and which contained ice and KCN (final concentration 20 mM). Cells were washed twice with ice-cold TE-KCN (Tris-HCl 10 mM pH 8.0-EDTA 1 mM-KCN 20 mM) resuspended in 2.5 ml 25% sucrose in Tris-HCl (0.25 M pH 8.0)-KCN (20 mM) and lysed by the successive addition of 0.5 ml lysozyme (10 mg  $\text{ml}^{-1}$ ), 1 ml EDTA (0.25 M, pH 8.0) and 4 ml of 10% Triton X-100 in Tris-HCl (0.05 M pH 8.0)-EDTA (0.063 M). The lysate was then centrifuged at 50,000*g* for 15 min at 0 °C to remove the bulk of chromosomal DNA and the supernatant fluid was added to 8 g CsCl plus 1 ml of ethidium bromide (10 mg  $\text{ml}^{-1}$ ) and was subjected to equilibrium centrifugation (at 36,000 r.p.m. for 60 h at 20 °C in the Beckman 50Ti rotor). Fractions were collected from the bottom of the centrifuge tube and the cold trichloroacetic acid precipitable radioactivity present in a 25- $\mu\text{l}$  aliquot of each fraction was determined. The lower panel shows the distribution of the  $^{14}\text{C}$  radioactivity (uniformly-labelled DNA) and the  $^3\text{H}$  radioactivity (pulse-labelled DNA). The upper panel shows the ratio of  $^3\text{H}$  to  $^{14}\text{C}$ . As has been observed for several circular DNA molecules<sup>1</sup>, the pulse-labelled material is found predominantly between the closed circular and open circular positions in the CsCl-ethidium bromide gradient. This indicates that replicative intermediates of the pSC101 plasmid have a closed-circular duplex component. It should be noted that some pulse label is found at a more dense region of the gradient than that where the closed circular plasmid DNA bands. The pulse-labelled DNA which sediments at this position represents a novel form of replicative intermediate which has also been observed recently by others (J. H. Crosa, personal communication; Y. Kupersztoch, personal communication) and which will be described in detail elsewhere (K.T., F.C., and S.N.C., in preparation; J. H. Crosa, L. K. Luttrupp and S. Falkow, in preparation). *b*, Electron microscopy of *EcoRI*-cleaved replicative intermediates. The material from the CsCl-ethidium bromide gradient represented by the hatched area in *a* was extracted with isopropanol

Coexisting independent pSC101 and ColE1 plasmids cannot provide in *trans* replication functions for each other<sup>7</sup>, suggesting that our previously observed replication of the composite plasmid in conditions of DNA polymerase I deprivation



to remove the ethidium bromide, dialysed 3 h at 4 °C against 1 l of TE buffer (two changes), treated with *EcoRI* (ref. 35) for 15 min at 30 °C and the DNA mounted for electron microscopy using the basic protein film technique<sup>36</sup> as described by Davis *et al.*<sup>37</sup> (ColD-CA23 (ref. 7), was used as an internal contour length reference). Grids were rotary shadowed with platinum-palladium and examined in a Philips EM201 electron microscope. *EcoRI*-cleaved molecules containing replication eyes or forks were photographed and measured and the fractional lengths of replicated and unreplicated segments were calculated and plotted as shown in *c*. Replicating molecules displaying whiskers<sup>38</sup>, which were assumed to have arisen by the process of branch migration during the isolation period, were ignored for the purposes of this experiment.



occurred by means of the pSC101 origin. To test this directly, we prepared replicating molecules of pSC134 from bacteria deficient in DNA polymerase I (*E. coli* W3110 *polA1*, ref. 14) and determined replication origin usage as described above. It was found that in such bacteria, the pSC134 hybrid utilises its pSC101 replication origin exclusively (Table 1) providing direct evidence that the pSC101 replication origin can function in the hybrid plasmid.

### Plasmid copy number

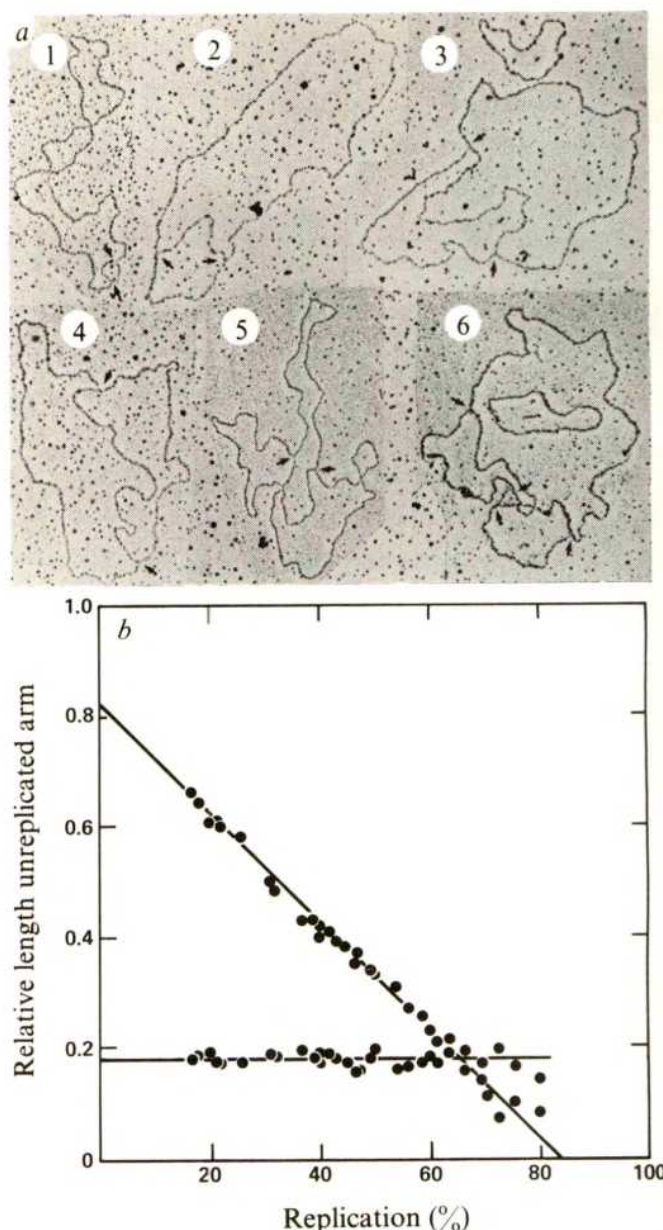
Although we have shown previously that both ColE1 and pSC101 replication functions are individually competent to replicate the entire pSC134 molecule<sup>7</sup>, it was not known whether one or both sets of functions are involved in the normal replication of the hybrid plasmid. Nordström, and Kool and Nijkamp have shown that for the R1 (ref. 15) and CloDF13 (ref. 16) plasmids, at least one product that controls the number of copies of a plasmid in a cell is encoded by the plasmid. Copy number seems to be a property of plasmid replication and may be regulated by a repressor of replication; copy number mutants frequently show altered incompatibility properties<sup>17</sup>. Moreover, the replication, copy number and incompatibility functions were shown to be clustered on a single *EcoRI*-generated DNA fragment in the case of the *F'-lac* and R6-5 plasmids<sup>18</sup>.

Since the replication functions of the pSC101 and ColE1 plasmids are apparently active only on the homologous replication origin (above, and ref. 7), exclusive use of the ColE1 origin during normal replication of the composite plasmid implies that ColE1 replication functions are being used. ColE1 and pSC101 have distinct copy numbers in logarithmically-growing cells (see below); thus determination of copy number of the composite plasmid should elucidate which of the parent plasmid copy number regulating factors is active in its replication.

Table 1 shows that under non-restrictive conditions of bacterial cell growth, the ColE1 and pSC101 plasmids have average copy numbers of 18 and 6 per genome equivalent, respectively. Coexistence of independently-replicating ColE1 and pSC101 plasmids in the same cell does not appreciably affect their relative copy number. In our experimental conditions, the pSC134 hybrid plasmid has a copy number of 16 and so exhibits the copy number characteristic of its ColE1 component. If ColE1 replication functions are inhibited by DNA polymerase I-deprivation, however, the composite replicon is maintained at a copy number typical of the pSC101 plasmid (Table 1). Thus, plasmid copy number correlates with and seems to be an indicator of the system that is being used to replicate the hybrid plasmid.

### Tetracycline resistance level

The plasmid copy numbers given above were determined by direct quantitation of plasmid DNA by density gradient centrifugation; they are supported by data showing the level of tetracycline (Tc) resistance expressed by the pSC134 hybrid and its parent pSC101 plasmid. The minimum inhibitory concentration (MIC) of Tc for cells carrying the pSC101 plasmid is about  $30 \mu\text{g ml}^{-1}$  whereas the MIC for cells carrying the pSC134 plasmid is about  $80 \mu\text{g ml}^{-1}$  (Table 1). The increased resistance specified by the pSC134 plasmid is not simply due to the presence of ColE1 in the cells since bacteria carrying pSC101 and ColE1 as independent replicons are resistant to only  $30 \mu\text{g ml}^{-1}$  of antibiotic. Furthermore, the observed increased resistance is not the result of changes in transcription/translation of the Tc-resistance gene in the pSC134 plasmid as a consequence of hybrid formation, since the hybrid confers resistance to only  $30 \mu\text{g ml}^{-1}$  of Tc in cells deficient in DNA polymerase I, which maintain pSC134 at the same copy number as pSC101. We conclude that the increased resistance to Tc of cells carrying the pSC134 plasmid must result from increase in the number of Tc gene



**Fig. 2** Plasmid pSC134 normally utilises its ColE1 replication origin. Replicative intermediates of plasmid pSC134 were isolated as described in Fig. 1 and either nicked by X irradiation to relax supercoiled molecules (a) or treated with *EcoRI*. The molecules were then examined by electron microscopy as described in Fig. 1. The small molecules present in 3 and 6 are ColD reference molecules. *EcoRI*-generated fragments containing replication eyes or forks were photographed and measured. All had contour lengths corresponding to that of ColE1. Fractional lengths of replicated and unreplicated segments of these molecules are displayed in b. It can be seen that the replication origin on these fragments is located 18% from one *EcoRI*-generated terminus and that replication proceeds away from this proximal terminus, as has been previously shown for ColE1<sup>11-13</sup>.

copies (plasmid copy number) from 6 to 16 per genome equivalent and reflects a gene dosage effect.

Unlike most other kinds of plasmid-borne antibiotic resistance, in which plasmid-specified antibiotic-modifying enzymes are produced, Tc resistance seems to result from plasmid-induced changes in bacterial cell membrane permeability<sup>19</sup>. Thus there has been discussion as to whether Tc resistance could exhibit gene dosage effects<sup>20</sup>, as has been observed for plasmid-specified proteins<sup>15, 21, 22</sup>. Our data and the recent results of



**Table 1** Replication origins and functions utilised by plasmid pSC134

<i>E. coli</i> strain	Replication origin usage ColE1	pSC101	Plasmid copy number	Level of Tc resistance
CR34 <i>polA</i> <sup>+</sup> (pSC134)	36	0	16	80
W3110 <i>polA</i> <sup>-</sup> (pSC134)	0	14	6	25
CR34 <i>polA</i> <sup>+</sup> (pSC101)	—	—	5	25
C600 <i>polA</i> <sup>+</sup> (ColE1, pSC101)	—	—	18, 5	30

Origin usage by pSC134 in cells either wild type (*E. coli* CR34, ref. 34) or deficient (JG112 = W3110 *polA*<sup>-</sup>, ref. 14) for DNA polymerase I was determined by electron microscopy of *Eco*RI-cleaved replicative intermediates prepared as in Fig. 1 (only a few replicating pSC134 molecules were observed in DNA prepared from strain JG112). The plasmid copy number is a minimum value of the number of plasmid copies per genome equivalent. Values obtained by centrifugation of Sarkosyl crude cell lysates to equilibrium in CsCl-ethidium bromide gradients<sup>7</sup> and by sedimentation of cleared lysates through neutral sucrose gradients<sup>7</sup> were in close agreement. The level of tetracycline (Tc) resistance exhibited by the different bacteria is expressed as a minimum inhibitory concentration (MIC) in  $\mu\text{g ml}^{-1}$ , as determined by spreading about 100 cells on nutrient agar plates containing the antibiotic at different concentrations. The copy number and Tc-resistance level characteristics of the pSC101 plasmid were comparable in *polA*<sup>-</sup> and *polA*<sup>+</sup> bacteria.

Clewell *et al.*<sup>23</sup> indicate that plasmid specified Tc resistance is affected by changes in gene dosage.

### Incompatibility of pSC134 with parent replicons

Elucidation of the relationship between use of a particular replication system by a plasmid and the plasmid's incompatibility properties was an important objective of the present study. Earlier studies of incompatibility between related plasmids have been restricted to replicons which have acquired distinguishing phenotypic traits by genetic recombination *in vivo* (ref. 24 for example). Although incompatibility of recombinant plasmids with the parent replicons or related plasmids has been demonstrated<sup>42,43</sup>, the propinquity of incompatibility properties to plasmid replication could not be determined. DNA cloning methods<sup>25</sup>, however, now enable the construction of marked plasmids with identical replication systems. A series of *Eco*RI restriction endonuclease-generated DNA fragments that code for different antibiotic resistance genes (Ap, Km, SuSm) have recently been isolated from antibiotic resistance plasmids, and have been individually inserted *in vitro* into the separate ColE1 and pSC101 replicons (refs 18, 22, 25, 26 and K.T., F.C., and S.N.C., in preparation). Using such constructed molecules, the clonal inheritance of plasmids known to have identical replication functions can easily be followed during incompatibility studies by means of their different antibiotic resistances.

Incompatibility studies were carried out for the pSC134 composite replicon using ColE1-Ap, ColE1-SmSu, pSC101-Km, and pSC101-Ap derivative plasmids. Our results indicate that pSC134 is incompatible with both parent plasmids, despite its normal use of the ColE1 replication functions and origin only. The rate of segregation of pSC134 and ColE1-SmSu (Fig. 3c) is comparable with the segregation rate observed with the ColE1-Ap and ColE1-SmSu plasmids (Fig. 3d). In contrast, the segregation rate observed between pSC134 and pSC101-Km (Fig. 3a) is much greater than between pSC101-Km and pSC101-Ap (Fig. 3b).

### Models for the control of initiation of DNA replication

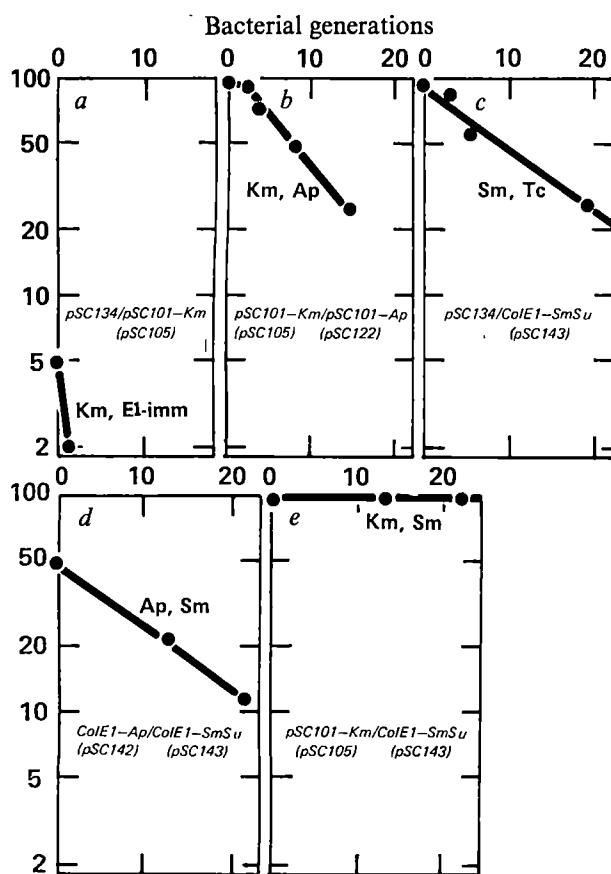
The studies reported here have used a specifically designed and constructed composite plasmid replicon for investigation of control mechanisms regulating plasmid replication, copy number, and incompatibility. Our results indicate that although the hybrid plasmid replicon pSC134 can use either of its two component replication systems, in normal conditions only its ColE1 replication functions and origin are used. The copy number of the composite plasmid is maintained at the ColE1 level. When the ColE1 replication functions are inhibited by DNA polymerase I deprivation, control of replication occurs by means of the origin and functions of the pSC101 component, and the copy number falls to a level typical of that plasmid. Moreover, although only the ColE1 replication system is

normally used by the composite plasmid, in such circumstances it is nevertheless incompatible with both of its parent replicons. These various features of replication of the pSC134 composite plasmid must be taken into account by any suitable model for control of the initiation of DNA replication.

The replicon model of Jacob, Brenner and Cuzin<sup>27</sup>, a positive control model, postulates that replication and maintenance of replicons takes place on cell membrane attachment sites. Replication occurs when the sites are duplicated and progeny molecules are subsequently distributed among daughter bacteria at cell division. According to this model, compatible plasmids have different membrane attachment sites, whereas incompatible plasmids compete for the same sites; incompatible plasmids initially present in the same cell thus segregate at cell division. Since both ColE1 and pSC101 replication origins and functions have been shown to be available for replication of the pSC134 composite plasmid, a prediction of the model of Jacob *et al.* is that each of the component systems should be used to replicate some plasmid molecules. Therefore, this model would predict that pSC134 should have a copy number similar to the additive plasmid copy number in cells carrying independently-replicating ColE1 and pSC101 plasmids, since each component replicon should be capable of producing its normal complement of copies whether or not it is physically linked to a second replicon. Finally, the model of Jacob *et al.* predicts that the pSC134 plasmid should be compatible with its pSC101 parent, since the hybrid replicon uses only its ColE1 replication origin and functions for normal replication, and thus should not compete for any pSC101-specific bacterial cell membrane replication sites. These predictions are not fulfilled by our experimental findings.

An alternative model involving negative control of initiation of DNA replication proposed by Pritchard, Barth, and Collins<sup>28</sup> postulates that a replication repressor substance is involved in the control of initiation of DNA synthesis. Replication is initiated when the repressor is diluted out to a critical concentration during the course of cell growth (that is, when a critical cell mass per replicon, the initiation mass, is achieved). If incompatible plasmids specify identical or similar initiation repressors, as required by this model, the total number of copies of the two plasmids could not exceed the usual copy number of one of them alone. Segregation of the two incompatible replicons would then occur at cell division. This model predicts that regardless of the replication functions used by the pSC134 hybrid plasmid, it should direct the synthesis of both types of initiation repressor, and thus should be incompatible with both parent plasmids (or derivatives thereof).

Implicit in the Pritchard *et al.* model is the expectation that a low copy number (high initiation mass) plasmid will be inhibited at low effective concentrations of its specific repressor, whereas greater effective concentrations of the repressor of a high copy number (low initiation mass) plasmid are required to inhibit its replication. Consequently, if two replicons such as ColE1 and pSC101 (which have different copy number) are fused, replication of the hybrid will be controlled by the ColE1 component<sup>29</sup>. Replication of this component will be prevented



**Fig. 3** Incompatibility properties of pSC134. The *in vitro* construction of the pSC101 derivatives pSC101-Ap (pSC122) and pSC101-Km (pSC105) which code for resistance to ampicillin and kanamycin, respectively, has been described<sup>18,25</sup>. ColE1-Ap (pSC142) was constructed by insertion of Ap-DNA isolated from pSC122 into the *EcoRI* site of ColE1. ColE1-SmSu (pSC143) contains an *EcoRI* fragment coding for resistance to streptomycin (Sm) and sulphonamide (Su) from R6-5 (K. T., F. C., and S. N. C., in preparation). Plasmids pSC134, pSC142, and pSC105 were introduced into *E. coli* K12 HfrC (strain W2252 kindly provided by A. Ganesan) by transformation<sup>26</sup>. After transformants had been purified by single colony isolation, their donor ability was determined using MS2 bacteriophage. These plasmids were then transferred by conjugation to *E. coli* K12 CR34 NaI<sup>r</sup> bacteria containing pSC105, pSC122, or pSC143. Transconjugants were selected on media containing nalidixic acid (50  $\mu\text{g ml}^{-1}$ ) and the combinations of antibiotics required to select both plasmids. Antibiotics were used at the following concentrations: Ap and Km 50  $\mu\text{g ml}^{-1}$ , Sm 25  $\mu\text{g ml}^{-1}$ , and Tc 10  $\mu\text{g ml}^{-1}$ . Transconjugant colonies which appeared on the double selection plates were used directly for segregation studies. Segregation of the plasmids was followed by picking colonies to flasks containing L-Broth plus nalidixic acid and allowing the bacteria to grow for about 25 generations in the absence of other antibiotics. At intervals samples were plated on nutrient agar. Colonies which appeared on these plates subsequently were toothpicked to nutrient agar plates containing appropriate antibiotics to determine which plasmids were present in the clone. The data plotted indicate the fraction of cells containing both plasmids. The segregation pattern thus obtained for the various plasmid combinations is shown in panels a-e. Because the pSC134 plasmid and pSC101 derivatives code for Tc resistance, the segregation of pSC134 in cells carrying pSC101 derivatives was monitored on colicin E1-containing media by means of the colicin E1-immunity (E1-imm) coded by pSC134 (colicin E1 was prepared and assayed as described in refs 40 and 41). In the panels, the first component of each plasmid pair is the incoming plasmid and the second component is the resident. With all plasmid combinations except the one represented in panel a, the incoming plasmid could be either member of the pair. The pSC105 plasmid, however, could not be introduced into bacterial cells already carrying pSC134. In a and d it can be seen that some segregation had already occurred in the clones selected before the beginning of the experiment. This is probably due to destruction of antibiotic in and around the clone by enzymes specified by one plasmid.

only when the hybrid plasmid reaches the high copy number required to produce effective inhibitory concentrations of the ColE1 replication repressor substance, whereas pSC101 replication is prevented by the pSC101 repressor substance at low copy number. Because the pSC101 repressor is always in excess when copy number of the composite plasmid is high, replication activity of the pSC101 component of the composite plasmid should be prevented so long as the ColE1 replication system is functioning. A second pSC101 replicon that is introduced into a cell carrying pSC134 (Fig. 3a) similarly should be prevented from replicating. Inhibition of the ColE1 replication system of the composite replicon results in a drop in plasmid copy number to a level where the pSC101 replication system is now able to act. When copy number of the pSC101 replicon is not forced up by its attachment to the ColE1 replicon (that is, when the two plasmids coexist autonomously in the same cell) pSC101 is able to maintain its own control of copy number and utilise its own replication origin and functions despite the presence of ColE1.

The replication properties of the pSC134 composite plasmid seem not to be explicable in terms of the positive control replicon model. The simplest interpretation of our results implicates a replication origin-specific, freely diffusible plasmid gene product that functions by negative control of DNA synthesis and, to that extent, our findings support the general model for DNA replication control proposed by Pritchard *et al.* The present studies do not, however, concern the other major aspect of this model, namely the suggestion that the timing of initiation of DNA replication is controlled by the process of dilution of the initiation repressor during cell growth. An adequate explanation for phenomena such as relaxed replication (for definition see ref. 7) of certain plasmids in non-dividing stationary phase bacterial cultures may require a more elaborate concept than that of inhibitor dilution.

Recent evidence indicates that discrete segments of DNA can be translocated from plasmid to plasmid by non-reciprocal recombinational events<sup>30-33</sup>. Such events could lead to the formation of naturally-occurring replicons with multiple replication origins and functions. Experimentally, such plasmids may appear to contain a single replication origin if their component segments are derived from replication systems that specify different copy number, as is pSC134. Conversely, we can speculate that replicons which are observed to use more than one origin during normal replication are derived from plasmids with similar copy number. Presumably this may occur by the recombination of genetically different replication systems which coincidentally specify a similar copy number, by recombination of closely related replication systems derived from different plasmids, or by duplication of the origin of a single plasmid.

Since the pSC134 hybrid plasmid can utilise either one of its replication systems to propagate itself in conditions that are restrictive for the other region of replication activity, it is a bifunctional replicon. Other such replicons can be constructed to contain dual replication regions derived from diverse biological sources; they are potentially highly useful for the development of DNA cloning systems for hosts other than *E. coli*. Moreover, the ability of the pSC134 plasmid to propagate in *E. coli* by using either of its replication systems now provides a valuable tool for the isolation of plasmid mutants that are defective in one of the pSC134 component replicons.

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# letters to nature

## Can 'invisible' bodies be observed in the Solar System?

MANY theories of the origin of comets predict that there are  $\sim 10^{11}$  comets in the Solar System; most of them, unfortunately, at such a distance that they can never be observed. Some theories go even further and predict that, in addition to these comets in the Oort cloud, the remains of a primaeval comet belt may still exist at a distance of  $\sim 50$  AU. This idea has been investigated by Hamid *et al.*<sup>1</sup>, who show (from an analysis of observed perturbations on Halley's comet) that the total mass of the comet belt must be  $\lesssim 1$  Earth mass. Even this relatively small mass would be enough for  $\sim 10^{10}$  comets—a significant fraction of the total of the whole Oort cloud. It has been noted that comets contain a relatively large fraction of 'heavy' elements, and it has been suggested<sup>2</sup> that the total galactic population of comets may be large enough to affect significantly the chemical evolution of the Galaxy by acting as a 'sink' for the heavy elements.

The absence of observations of strongly hyperbolic orbits places a limit on the total mass of interstellar comets, but there remains the possibility that a great many comets may be permanently bound to their 'parent' star, and will thus always remain unobserved. Whipple<sup>3</sup> has considered this possibility and concludes that a mass  $>0.01M_{\odot}$  could be distributed as comets in the inner region of the Oort cloud (from 50 to  $\sim 20,000$  AU) and continue to remain undetected by present methods. It is clear that techniques based on the gravitational effect of hypothetical comets on observed ones are not yet sufficiently well developed to limit speculation, and it is my purpose to suggest a way in which this might be done.

Consider a 'boulder' in the Solar System of radius  $R$  and geocentric distance  $d$ . Its angular diameter is  $\theta = 2R/d$ ; similarly, the angular diameter of a star is  $\theta_* = 2R_*/d_*$ . The boulder can occult the star if  $\theta > \theta_*$ , that is if

$$Rd_*/R_*d > 1 \quad (1)$$

If the geocentric transverse velocity of the boulder is  $v_t$ , it will seem to move its own diameter in a time,  $\tau$ , where  $\tau = 2R/v_t$ .

The boulder therefore 'sweeps' a narrow track on the celestial sphere at a rate

$$\dot{A} = \theta^2/\tau = 2Rv_t/d^2 \quad (2)$$

Should there be  $n$  such boulders per steradian the whole sky can be considered to have been swept after a time  $T$ , where  $nAT \simeq 1$ , giving

$$T \simeq d^2/2nRv_t \quad (3)$$

If one is able to monitor  $n_*$  stars continuously, each satisfying the criterion (1), on the average one should observe an occultation once every

$$T' \simeq d^2/2nn_*Rv_t \quad (4)$$

As an example, we shall consider the possibility of detecting the hypothetical comet belt at  $\sim 50$  AU. To obtain a rough estimate of  $T'$  we shall suppose that the belt consists of  $N$  identical comet nuclei, each of radius  $R$  and density  $\rho$ . If the upper limit to the mass of the belt is  $M_u$  we have

$$N \lesssim M_u/(4/3)\pi\rho R^3 \quad (5)$$

One would expect the comets to show some concentration towards the ecliptic, and as a rough approximation we shall assume that the  $N$  comets are distributed over 2 sr of the celestial sphere. This means that  $n \simeq N/2$ , or

$$n \lesssim \frac{1}{2} M_u/(4/3)\pi\rho R^3 \quad (6)$$

then, from equation (4)

$$T' \gtrsim 4\rho R^2 d^2/n_* M_u v_t \quad (7)$$

For  $v_t = 10 \text{ km s}^{-1}$ ,  $R = 3 \text{ km}$ ,  $d = 50 \text{ AU}$ ,  $M_u = 5 \times 10^{24} \text{ kg}$ , and  $\rho = 10^3 \text{ kg m}^{-3}$ , we find

$$T' \gtrsim 4 \times 10^7/n_* \text{ s} \quad (8)$$

It is of interest to estimate the number of stars,  $n_*$ , that could be successfully monitored using existing technology. Perhaps the most suitable detector for the purpose is a SIT Vidicon

system, which scans a large area with high sensitivity and good time resolution. Noise in the system arises primarily from photon statistics, and for the following estimate of  $n_*$  it will be assumed that this is the only source of noise. Assuming that a 0 mag star produces  $\sim 1,000$  photons  $\text{\AA}^{-1} \text{cm}^{-2} \text{s}^{-1}$ , and that the photocathode has a bandwidth of  $3,000 \text{\AA}$  with a mean quantum efficiency of 15% (for example S-20), it can be shown that the contribution to the signal in a 1 m telescope from a 16 mag star is  $\sim 140$  photon events per 0.1 s. Taking the sky background to be equivalent to one 21 mag\* per square second of arc, and the resolution of the vidicon to be  $10''$ , we have  $S_* \simeq S_B$ . The total signal therefore consists of  $\sim 280$  photon events per 0.1-s interval, of which  $\sim 140$  on average come from the star. A fairly safe criterion for the occurrence of an occultation would be to count an 'event' each time the signal in any 0.1 s interval falls below that corresponding to 160 photon events. (Assuming that the photon events obey a Poisson distribution, the probability of such a fluctuation would be  $< 10^{-13}$ ; whereas equation (8) shows that the probability of an occultation is  $\sim 2.5 \times 10^{-9}$  per 0.1 s.) The statistics could be greatly improved for the case of occultations lasting longer than 0.2 s. A vidicon tube consisting of a  $300 \times 300$  array of resolution elements, each with the stated resolution, will be able to monitor  $\sim 0.7$  square degrees of sky which, considering stars brighter than 16 mag will contain  $\sim 1,000$  stars. With  $n_* = 1,000$ , the waiting time between occultations reduces to  $\sim 11$  h. One consequence of the use of such a technique would be that  $M_u$  would continually decrease if no occultation was seen. It should be emphasised that the figure of 11 h observing time per 'event' could almost certainly be greatly improved if an instrument was purpose built for the detection of occultations.

The above is meant only to illustrate the possibilities for such an observational technique. A complete theory would have to be statistical in nature, and the interpretation of a particular event would depend on the distributions of  $R$ ,  $d$ , and  $v$ , that one might expect to encounter. One would like, for example, to be able to distinguish between a boulder at 1 AU and a comet nucleus at 100 AU. If the detector system could be made sufficiently sensitive to be able to record 'partial' occultations, the detection of a variety of interplanetary debris may be possible and, in particular, the detection of comets at distances  $\gg 50$  AU would be possible. The technological problems involved in the design and construction of such a detector promise to be large, but should not be insurmountable. In view of the many important results likely to come from such a programme—benefitting not only cometary astronomy—it is to be hoped that someone will investigate the further possibilities of 'occultation' astronomy.

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## Third discontinuity in the Vela pulsar period

THE period of the Vela pulsar, PSR0833-45, decreased discontinuously by approximately two parts in  $10^6$  near the beginning of October 1975, the third such event observed for this pulsar. The first<sup>1,2</sup> occurred in March 1969, and the second<sup>3</sup> in August 1971.

Since May 1975 pulse arrival-time data have been recorded for a number of pulsars at the NASA Deep Space Station,

Tidbinbilla, located near Canberra, Australia, with an average spacing between observations of  $\sim 10$  d. A 26-m diameter antenna is being used at an observing frequency of 2,295 MHz. For the Vela pulsar 25 integrated pulse profiles are recorded on magnetic tape during an observation lasting  $\sim 20$  min. Typically, data from such an observation give a mean arrival-time having an uncertainty of 20  $\mu$ s. Propagation times to the Solar System barycentre are computed using an ephemeris provided by I. I. Shapiro of the Massachusetts Institute of Technology. As data have not been recorded for a full year, the pulsar position cannot yet be computed from the arrival-time data; the Molonglo position, RA 08 h 33 min 39.44 s, dec  $-45^\circ 00' 10.2''$  (ref. 4), was assumed.

Data recorded between 21 May 1975 and 25 September 1975 can be fitted quite closely by a parabolic curve; the parameters obtained from this fit are given as the pre-jump data in Table 1, where the errors quoted are dominated by the uncertainty in pulsar position. Significant residuals from the fitted curve, apparently resulting from intrinsic variations in the pulsar period, are observed. The r.m.s. residual for the 127-d period including 13 independent observations, is 165  $\mu$ s, large compared to the 20  $\mu$ s observational uncertainty. This pre-jump period agrees within a few parts in  $10^7$  with that extrapolated from parameters determined after the August 1971 event<sup>3</sup>, implying that no similar jump occurred between then and May 1975.

Pulse arrival times obtained on and after 15 October do not lie on the parabola extrapolated from these previous data. A least-squares fit of a parabola to data recorded from 15 October to 14 November gives the post-jump parameters listed in Table 1. Based on extrapolation of the pre-jump data, the period at JD 2442700.5069 would have been  $0.089\,234\,889\,53 \pm 15$  s. The magnitude of the period change was therefore  $175.6 \pm 0.2$  ns, corresponding to  $|\Delta P|/P = \Delta\Omega/\Omega = 1.97 \times 10^{-6}$ , and to a phase drift of about 1.9 cycles  $\text{d}^{-1}$  with respect to the pre-jump period. Because of this large drift it is not possible (by extrapolating the post-jump data backwards) to determine on which day the period decrease occurred. It can only be said that it occurred between JD 2442680.64 (September 25) and JD 2442700.51 (October 15).

Table 1 Timing parameters for the Vela pulsar

Pre-jump data	(JD 2442553-2442680)
Period	$0.089\,233\,318\,26 \pm 7$ s (A.1)
$dP/dt$	$(124.096 \pm 0.010) \times 10^{-15}$ s $\text{s}^{-1}$
Epoch (JD)	2442553.9595
Post-jump data	(JD 2442700-2442731)
Period	$0.089\,234\,713\,88 \pm 7$ s (A.1)
$dP/dt$	$(125.03 \pm 0.01) \times 10^{-15}$ s $\text{s}^{-1}$
Epoch (JD)	2442700.5069

The data in Table 1 also show that there was an increase in the rate of change of the period of  $(0.93 \pm 0.02) \times 10^{-15}$  s  $\text{s}^{-1}$ , apparently coinciding with the decrease in the period. The corresponding value of  $\Delta P/\dot{P} = |\Delta\Omega|/\dot{\Omega}$  is  $7.5 \times 10^{-3}$ . Because of possible systematic variations in the period and its derivatives following the jump, and the superimposed random irregularities, the post-jump parameters must be considered preliminary values only.

The parameters of this third jump are remarkably similar to those of the previous jumps in spite of the fact that the interval between the second and third jumps is over 60% greater than that between the first and second. Values of the period decrease for the 1969, 1971 and 1975 events are, respectively,  $\sim 200$  ns, 178.6 ns and 175.6 ns. For the 1969 event the period derivative increased by  $\sim 1.00 \times 10^{-15}$  s  $\text{s}^{-1}$ , compared to  $0.93 \times 10^{-15}$  s  $\text{s}^{-1}$  for the 1975 event. The increase in  $dP/dt$  for the 1971 event was comparable.

Further analysis of the variation in period for before and after this third discontinuity will be undertaken when additional post-jump data are available.



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## B-emission stars and X-ray sources

SEVERAL transient X-ray sources with decay times of the order of weeks to months have been reported<sup>1</sup>; the discovery of four new sources of this type, A1118-61 (refs 2 and 3), A0535+26 (refs 4-6), A1742-28 (P. W. Sanford, personal communication), A1523-61 (ref. 7), in less than 1 yr of operation of the X-ray satellite Ariel V, indicates that these objects are rather common. Important for the understanding of this phenomenon is the optical identification of the sources: it has been proposed that, A1118-61 and A0535+26, coincide with bright B stars with emission lines ('Be' stars)<sup>8,9</sup>, while A0621-00 is identified with a completely different object, which showed a nova-like behaviour optically, simultaneous with the X-ray outburst<sup>10</sup>. Here we discuss X-ray emission from systems containing Be stars.

Be stars are appealing candidates for X-ray transients for the following reasons: first, a number of these stars are single-line spectroscopic binaries with periods of  $\sim 10$  d (ref. 11). It is suggested that many Be stars have an evolved, invisible, close companion. The very rapid rotation of these stars is then naturally explained by mass exchange from the envelope of the presently invisible (though initially more massive) companion. Second, evolutionary considerations<sup>12</sup> show that if some mass loss from the system occurs during the first stage of mass transfer, compact stars (neutron stars or black holes) can be formed as companions of main-sequence stars of masses as low as  $8-10 M_{\odot}$ . This corresponds to main-sequence spectral type as late as B3-B4 (Be stars are indeed main-sequence stars, and are generally found in the entire B-type spectral region). The lifetime of main-sequence stars of  $8-10 M_{\odot}$  is  $\sim 2 \times 10^7$  yr. Third, Be stars characteristically show a central absorption component in the Balmer lines, indicating the presence of circumstellar material. It is generally thought that this matter is ejected from the equatorial regions where, because of the extremely rapid rotation of the Be stars, the effective gravity is almost zero. The emission features vary over time scales of 1-100 yr (ref. 11).

We propose that sudden variations in the rate of mass ejection from Be stars in the presence of a compact companion could produce transient X-ray emission, which might recur over a period of years, like the observed optical emission of Be stars. The companion star should be a neutron star, rather than a white dwarf, because the spectrum of the transients is rather hard ( $\alpha = 1.0$  for pulsating sources, and 2-4 for the others) and the luminosity rather high ( $\approx 10^{37}$  erg s<sup>-1</sup>). The total amount of accreted matter required for an outburst is, for a neutron star,  $\Delta M \approx 10^{23}$  g, which is only a few per cent of the amount of mass in the envelopes of Be stars required for producing the observed emission lines.

Two of the transients (A1118-61 and A0535+26) vary periodically with very stable periods of 405 s and 104 s, respectively. If these are rotation periods for neutron stars they

are very slow, but nevertheless conceivable, since the lifetime of these systems can be  $> 10^7$  yr. On such a time scale the rotation could have been slowed down by the propeller mechanism<sup>13</sup>. The X-ray binary Vela X-1 is a slow pulsar ( $P = 283$  s (ref. 14)), and this supports the idea that these slowly pulsing transients might be neutron stars in binaries with orbital periods of the order of days. (Early B stars are observed<sup>15</sup> to have weak winds that can provide sufficient braking for a neutron star companion on this time scale<sup>16</sup>.)

Other systems identified with objects optically similar to Be stars are 3U0352+30 (X Per) and Cen X-3 (Krziminski's star). X Per has been classified as an extremely rapidly rotating (peculiar) B0Ve star<sup>17</sup> and Krziminski's star is an O6V-III emission star (rather close to the main-sequence, and not a clear Of star, since strong 4640 Å and 4686 Å emission is absent<sup>18</sup>). In two other cases, 3U1145-61, 3U1223-62 there are Be stars in the error boxes (HD 102567, Wray 977). If the identification with X Per is correct, 3U0352+30 has an X-ray luminosity of  $\sim 10^{33}$  erg s<sup>-1</sup>, which seems to be modulated with a period of 22 h (N. E. White, P. W. Sanford and K. O. Mason, unpublished). It is a remarkable fact that the maximum (break-up) rotational velocity for a B0V star corresponds to a rotation period of  $\sim 1$  d. We therefore suggest that the X-ray period represents the orbital period of a binary system, and that X Per rotates synchronously with this orbital period. The low X-ray luminosity would arise from either a low rate of mass transfer or a source which is almost completely buried by a supercritical mass accretion rate<sup>19</sup>. Such weak sources are measurable at the Uhuru sensitivity, only to within 0.5-1 kpc. It is, therefore, possible that 3U352+30 is a 'low' state for a transient X-ray source, and is observable only because it is close ( $\sim 170$  pc).

It seems important that the X-ray behaviour of Cen X-3 is in many respects similar to that of the pulsating transient X-ray sources, in that it turns 'off' for as much as a month at irregular intervals; when it turns on, its X-ray spectrum shows intense low-energy absorption<sup>20</sup>, very similar to the peak behaviour of the transients seen by Ariel V. A further similarity with these two transients is that Cen X-3 shows regular X-ray pulsations (though with a much shorter period ( $\sim 5$  s)).

If our suggestion that a class of X-ray transients is associated with Be stars is correct, it follows that the total number of these types of transients in a quiescent state is given by the number of Be transients per year ( $\sim 4$ ) divided by the frequency of active periods in Be stars ( $\sim 10^{-1}$  yr<sup>-1</sup>). Therefore—on the binary model—Be transients in a quiescent state should be approximately as numerous as steady galactic X-ray sources.

*Note added in proof:* After submission of this paper, 3U0352+30 (X Per) and 3U1223-62 (Wray 977) were discovered to be slow pulsars with periods of 13.924 and 11.64 min, respectively<sup>21,22</sup>. This seems a strong confirmation of our above suggestion (based on their association with Be Stars) that these sources are related to the slowly pulsating transients and to sources like Cen X-3.

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## Does Mercury have a molten core?

THE Mariner 10 mission has discovered a magnetic field associated with the planet Mercury<sup>1</sup>. The preferred explanation of the source of this field based on these observations, is an internal dynamo<sup>2</sup>. A necessary condition for such a dynamo is the existence of an electrically conducting liquid region within the planet. In this letter, we investigate whether or not Mercury could contain a molten metallic core.

Internal temperature distributions for a solid, undifferentiated Mercury have previously been calculated by Majeve<sup>3</sup>. Siegfried and Solomon<sup>4</sup> considered the effects of melting and fractionation. They concluded that Mercury would have a metallic core fully differentiated from the silicate mantle if the uranium and thorium abundances are taken from the cosmochemical model of Lewis<sup>5</sup>. According to Siegfried and Solomon, such models predict a present-day temperature profile rising from the surface temperature to the melting curve in the upper 300 km, a molten mantle and a slowly cooling core either at or below the melting curve of iron. It has been pointed out<sup>6,7</sup> that such a thermal constitution would prevent dynamo generation. We have extended the work of Reynolds *et al.*<sup>8</sup> and Fricker *et al.*<sup>9</sup> to study the possible thermal evolution of a metallic core in Mercury. The calculations involve the solution of the equation of heat conduction for a spherically symmetric body with internal heat sources. Modifications have been incorporated to take into account the latent heat of fusion, and the redistribution of radioactive heat sources as a consequence of melting. The numerical methods used follow the method described in ref. 8. For the internal structure of Mercury, the work of Reynolds and Summers<sup>10</sup> was used. Following Lewis<sup>5</sup>, it was assumed that the condensation of U, Th and Fe was complete at the orbit of Mercury and that, like other volatile and chalcophile elements, K can be neglected. For a model of Mercury with a metal weight-fraction of 66%, an average U concentration of  $2.25 \times 10^{-8} \text{ g g}^{-1}$  as estimated by Wasserburg *et al.*<sup>11</sup> for the Earth would correspond to a U content of  $4.4 \times 10^{-8} \text{ g g}^{-1}$ , if the Fe/U ratio of the Earth is applied. This value together with a Th/U ratio of 3.7 has been used. The calculated heat conduction for the silicate material relies on the data given by Schatz and Simmons<sup>12</sup> for the mantle material of the Earth; it includes the contributions of lattice conductivity and radiative transfer. The conductivity of iron was taken as  $2.5 \times 10^8 \text{ erg cm}^{-1} \text{ K}^{-1} \text{ s}^{-1}$ . The heat capacity of the silicate material was assumed to be  $1.2 \times 10^7 \text{ erg g}^{-1} \text{ K}^{-1}$ , that of iron  $0.6 \times 10^7 \text{ erg g}^{-1} \text{ K}^{-1}$ . For the bulk melting temperature of the silicates, the melting curve of diopside (Fig. 1) was adopted<sup>13</sup>. The surface temperature was held constant at a value of 75 °C (ref. 14).

Interpretation of the recent Mariner results suggests that extensive igneous activity has occurred on the surface

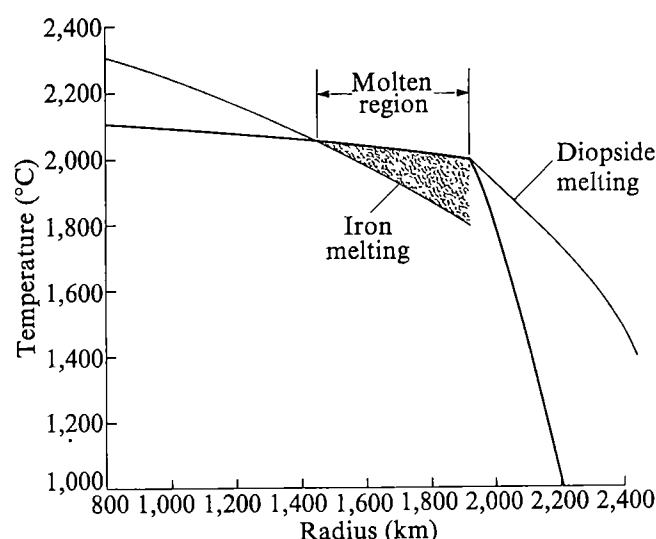


Fig. 1 The heavy curve is the calculated temperature profile of Mercury  $4.6 \times 10^9$  yr after core formation. The diopside and iron melting curves are shown. The molten core region is  $\sim 500$  km thick.

of Mercury: there is evidence that global differentiation occurred very early in the planet's history, resulting in the formation of a metallic core<sup>15,16</sup>. The high initial temperatures required for an early differentiation process have evidently also prevailed in the other terrestrial planets and the moon, as well as in the relatively small meteorite parent bodies<sup>8,17,18</sup>. Therefore, the calculation was started with an initial temperature distribution corresponding to the melting curve of iron<sup>19,20</sup>, and the core was assumed to have formed essentially coincident with planetary formation,  $4.6 \times 10^9$  yr ago. As a result of the melting and differentiation process, the radioactive heat sources are assumed to be concentrated in the outermost layers. Other assumptions regarding the initial state of the planet tend to result in higher interior temperatures at the present time, since core formation is delayed and less time is available for cooling.

The boundary between the iron core and the silicate mantle at a pressure of  $7 \times 10^9 \text{ Pa}$  ( $1 \text{ Pa} = 10^{-5} \text{ bar}$ ) coincides with a substantial difference in melting temperatures: the melting curve of diopside is  $\sim 200$  °C higher than the melting curve of iron at this point. This difference holds also for other silicate materials, which are depleted in volatile and chalcophile elements<sup>21</sup>. In addition, the presence of Ni, or of other minor constituents, in the core, would tend to lower the assumed iron melting curve. The core-mantle boundary separates two contrasting thermal conductivity regimes. The differences in melting temperatures and transport properties has a crucial role in the thermal evolution of the interior of the planet. Because of the higher melting temperature in the deep mantle and the lower conductivity of the mantle material in comparison with iron, the core-mantle boundary represents an effective heat transfer barrier in the absence of efficient solid convection in the mantle. This barrier permits temperatures in the outer core to rise above the melting curve of iron, with a corresponding reduction of the temperature gradient at the core-mantle boundary. If the barrier did not exist, the heat flux from the interior would be at least equal to the product of the iron thermal conductivity and the melting curve gradient, a value sufficient to solidify the entire core in  $< 2 \times 10^9$  yr. The models of Siegfried and Solomon<sup>4</sup> neglect the melting temperature discontinuity, and therefore predict solidification of the core and a large molten region in the mantle.

The temperature profile predicted by the thermal history calculations for  $4.6 \times 10^9$  yr, using the above initial conditions and parameter values, is given in Fig. 1. For our model, the inner 1,400 km of the core would now be solid, while the outer 500 km of the core remains molten. This model, therefore, fulfils a necessary requirement for a dynamo: at least a portion of the core is molten. It is also consistent with the apparent lack of recent degradation of surface features by magmatic activity, since the mantle is solid.

We wish to emphasise the following: the result that a molten shell exists now, and is a direct consequence of the discontinuity in melting temperatures at the core-mantle boundary. This result is independent of the density of planetary heat sources and the particular initial conditions imposed, as long as a molten core once existed and in so far as solid convection in the mantle can be ignored (see below). Core formation later than  $4.6 \times 10^9$  yr ago, retention of radioactive heat sources in the core or deep mantle, and the action of other energy sources (such as tidal dissipation or electrical heating) would all result in a larger present-day molten region than in our model.

The calculations described here do not include the effects of solid convection in the mantle, which could solidify the core under certain circumstances. Cassen *et al.*<sup>22</sup> have discussed this aspect of the problem, and find that it is necessary that a density of heat sources at least comparable with the Earth's mantle-wide average be retained throughout Mercury's mantle if the core is to remain molten in the presence of solid convection. (This requirement is additional to the melting temperature discontinuity at the core-mantle boundary.)

Finally, we note that our calculated temperature gradients in the molten region of the core are subadiabatic. Therefore, although a dynamo is possible in this model, it would have to be driven mechanically, rather than by thermal convection. Gubbins<sup>23</sup> has discussed the energy requirements of a convective dynamo.

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## Mid-latitude ionospheric scintillation fading of microwave signals

MEASUREMENTS of the amplitude of microwave (1,550 MHz) signals from the NASA ATS-5 geostationary spacecraft were made from December 1973 to July 1974. These data have been analysed for diurnal, geomagnetic and seasonal variations, and also for dependence on the boundary of the high latitude scintillation region in the direction of the Equator.

The signal to the spacecraft, at a frequency of 1,650 MHz, was transmitted from the DOT/TSC/Westford Propagation Facility at Westford, Massachusetts (42.6°N; 71.5°W, geographic). It was produced as follows: a 2-W signal from an L-band exciter was used to drive a 200-W travelling wave amplifier. The signal was carried through 15.2 m of rigid feedline to a 4.6-m diameter parabolic reflector antenna whose feed was such that right-hand circular polarisation was transmitted. A high power narrow-band filter after the output of the final amplifier insured that no out-of-band spurious emissions were transmitted.

This signal was frequency shifted by the L-band transponder of the spacecraft, in the narrow-band frequency translation mode, to a frequency of 1,550 MHz and re-emitted. The power level in the uplink was high enough to saturate the ATS-5 L-band transponder by > 7 dB thus assuring that the amplitude variations on the downlink were 'safe' from uplink fading contamination by 7 dB.

The signal beam crossed the 350-km level in the ionosphere at 30.1°N, 75.8°W (near Philadelphia) because the ATS-5 had a subsatellite longitude of 105°W. The invariant latitude  $\Lambda$  was 54°N (ref. 1) ( $\Lambda = \cos^{-1}(1/L)$ , where  $L$  is McIlwain's coordinate<sup>2</sup>).

The downlink signal was then received at the Westford facility on three independent receiving systems where its amplitude was recorded in both analogue and digital form.

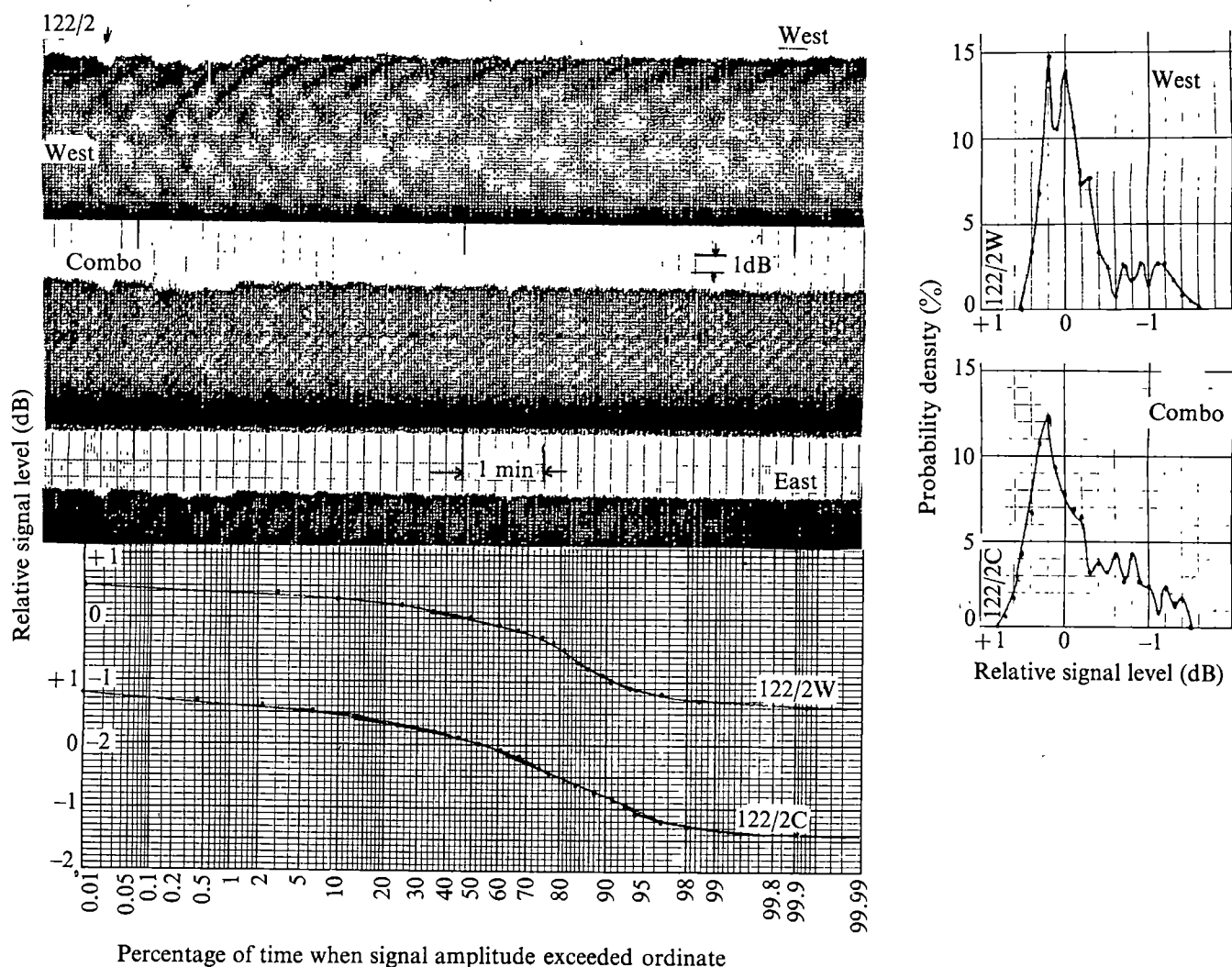
Each system consisted of a 3.1-m diameter parabolic reflector antenna with a preamplifier mounted at its spiral feed so chosen that right-hand circular polarisation was received.

Two of the receiving systems converted to a frequency of 30 MHz and then to 10 MHz, the signal from the other receiving system was converted to 136 MHz, then to 55 MHz and finally to 10 MHz. These 10-MHz signals were each filtered with a 30-kHz crystal filter, re-amplified and square-law detected. A portion of each filtered 10-MHz signal was also fed to three vector voltmeters which were used as narrow-band receivers with their 10-MHz reference signal coming from a frequency standard at the station. The vector voltmeters had a bandwidth of 1 kHz and employed a square-law detector following the narrow-band filter.

The 30-kHz detector voltage from each of the three receivers was recorded on an analogue strip-chart simultaneously with the detector voltage from each of the three vector voltmeters on a 6-channel strip-chart recorder. The calibration of the recorder was simplified by the identical calibration of the three vector voltmeters and of the three 30-kHz detected voltages.

Three independent receivers, each with a narrow- and wide-band mode, were used on the received signal to assure that the drift caused by gain instabilities could be isolated from shifts of frequency in the spacecraft's transmitter. During the measurement programme, a frequency drift in the spacecraft's transmitter was never observed.

The three detected voltages were digitised, preprocessed and recorded on magnetic tape for later computer processing<sup>3</sup>. An example of the analog data and the computer calculated statistics for April 1, 1974 are presented in Fig. 1. The strip-charts of the 30-kHz detectors are shown.



**Fig. 1** Example of analog data and computer calculated statistics for scintillation on April 1, 1974. ATS-5 subsatellite longitude:  $105^{\circ}\text{W}$ , Westford elevation angle:  $30.5^{\circ}$ ; Azimuth angle:  $224^{\circ}\text{SW}$ . The reason for the spiked fence appearance of the strip-chart recording is that the ATS-5 spacecraft was spinning with a period of 785 ms and thus traced out its L-band antenna pattern during each sweep. DOT/TSC/Westford propagation facility: NASA/ATS-5 spacecraft in NBFT Mode; WPF uplink at 1651.270 MHz; Downlink at 1550.250 MHz at  $S/N = 30$  dB;  $BW = 1$  kHz; median signal level =  $-117.5$  dBm (West) and  $-120.0$  dBm (Combo); Start time = 1415 EDT; file duration = 12 min.

Note how all three channels fade simultaneously. The calculated probability densities<sup>4</sup> for two of the three channels are also presented, as well as the cumulative amplitude distribution for the 12-min measurement interval.

In each of the 2,500 12-min data samples the best channel (that with amplitude drift  $< 0.2$  dB) was analysed. The 12-min data samples were statistically analysed to determine mean values, root-mean-squares (r.m.s.), probability density functions and probability distribution functions. The value of the 12-min distribution function at the 90% level was termed a 'scintillation index'. These 12-min 'scintillation index' values were grouped into 1-h and 3-h intervals to form '1-h scintillation index' (1-h SI) and '3-h scintillation index' (3-h SI) values, respectively.

The 3-h SI values were associated with the corresponding planetary magnetic index values which are computed for standard 3-h intervals<sup>5</sup>. The results, presented in Fig. 2 show that the higher values of magnetic activity are associated with the higher values of the 3-h SI.

Figure 3 presents the diurnal variations of the microwave ionospheric scintillation as expressed by the 1-h SI. For low  $Kp$  ( $Kp < 4$ ) there seem to be slight maxima at 1800, 2000 and 0000 LMT. For high values of  $Kp$  ( $Kp > 4$ ) there are slight maxima at 1800 and 0000 LMT.

Also plotted on Fig. 3 is the latitudinal variation of the mid-latitude scintillation boundary, as determined by

Kersley *et al.*<sup>6</sup>. The appropriate microwave scintillation data ( $Kp < 4$ ) do not seem to be directly related to the position of the scintillation boundary as determined by the measurements at 40 MHz. There is, however, a well-defined peak during the period when the statistically averaged scintillation boundary crosses the  $54^{\circ}$  parallel. Although the two sets of data were taken at different phases of the sunspot cycle and different geographical longitudes, the data do fit together.

During the solstices the data looked relatively calm with only a few r.m.s. signal level fluctuation measurements  $> 0.4$  dB during December and January, or June and July. During the vernal equinox, however, the r.m.s. signal level did have values in the  $0.4$ – $0.6$  dB range as would be indicated by the conclusions of Oksman and Taurianien<sup>7</sup>.

The following are our conclusions so far: first, ionospheric scintillation at microwave frequencies does exist at mid-latitudes. Second, of the 250 h of observations analysed, 10.2% were found to be undisturbed with a r.m.s. fluctuation level of  $\leq 0.22$  dB. Also, 0.84% of the observation periods were found to have disturbed propagation conditions with the 90% levels exceeding 1 dB which corresponds to a  $\sim 2$  dB peak-to-peak fluctuation level. Third, the largest peak-to-peak fluctuation that was observed on the strip-chart recording was 3 dB. Likewise, the smallest r.m.s. fluctuation level for an observing period was 0.15 dB.



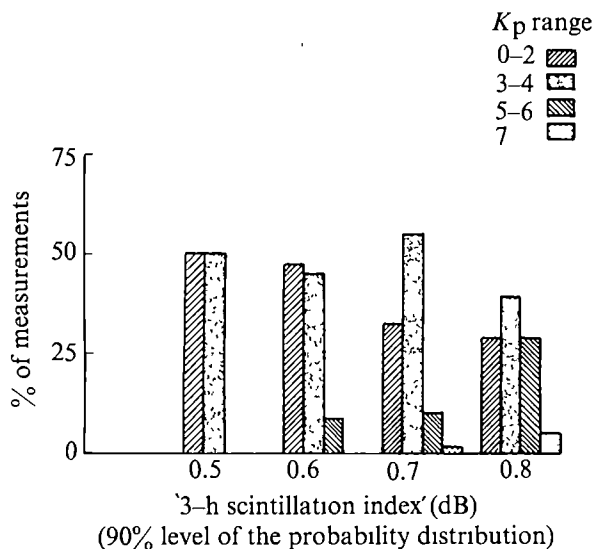


Fig. 2 Comparison of 3-h SI with the planetary magnetic index,  $K_p$ , (December 1973–July 1974).

Fourth, mid-latitude ionospheric scintillation is related to magnetic activity, in that higher levels of magnetic activity are associated with higher levels of microwave scintillation. Fifth, mid-latitude scintillation has some diurnal variations<sup>8</sup>. Sixth, some seasonal variation was indicated, with the smallest scintillation activity during the winter solstice, and the maximum scintillation activity around the vernal equinox.

Fading of the amplitude of microwave signals has been reported for equatorial regions<sup>9–11</sup> and for auroral or polar

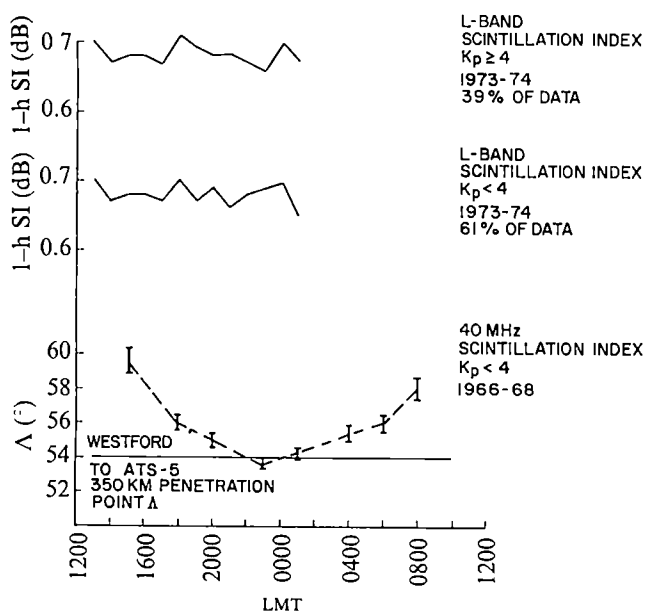


Fig. 3 Diurnal variation of microwave ionospheric scintillation for two ranges of planetary magnetic index shown with the scintillation boundary determined by Kersley *et al.*<sup>6</sup> from 40-MHz transmissions from the BE-B satellite.

regions<sup>12</sup>. Which has led to studies of the spectrum of ionospheric irregularities particularly in the F-region from *in-situ*<sup>13</sup> and ground-based measurements<sup>14</sup>. As far as we are aware, the observations reported here represent the first cases of scintillation fading due to F-region irregularities for frequencies in the microwave band at mid-latitudes.

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## Long conductors as antennae for gravitational radiation

To date, the antennae used in the search for gravitational radiation have been mechanical in nature, that is they involve the detection of small displacements or strains in mechanical systems. Here we demonstrate that a conceptually simple system of two long conducting wires of different metallic composition constitutes, in principle, a non-mechanical antenna for gravitational radiation, and that if the conductors are sufficiently long such a system may be of practical use. Very briefly, the principle of operation of the system is that gravitational radiation produces a greater force on the ions in a metal than on the electrons, which, to preserve charge neutrality, necessitates a compensating electric field which is, in principle, detectable.

The effect of a constant gravitational field on a conductor has been studied by a number of authors; we note in particular the theoretical work of Barnhill and Schiff<sup>1,2</sup>, and of Dessler, Michel, Rorschach, and Trammell<sup>3</sup>. The result of this work is the prediction that a vertical electric field will exist inside and in the vicinity of such a conductor with magnitude

$$E = \Lambda mg/e \quad (1)$$

Here  $m$  is the mass of an ion in the metal,  $e$  is the charge of the electron,  $g$  is the acceleration due to gravity, and  $\Lambda$  is a constant representative of the metal, typically  $\sim 0.1$ – $1$  (ref. 3). We may understand this result qualitatively as follows: the gravitational force on the ions is  $mg$ ; on the much lighter electrons it is thousands of times less. To preserve charge neutrality it is necessary that the electrons experience an electric force  $eE$  of the same order of magnitude as  $mg$ . Equation (1) expresses the equality of these forces.

If we consider a conductor in rapid rotation it is evident that similar considerations will apply if we replace the gravitational force  $mg$  by the centrifugal force, which can be made quite large by the use of high rotation rates.

$$mg \rightarrow m\omega^2 l; E = \Lambda m\omega^2 l/e \quad (2)$$

Here  $\omega$  is the rate of rotation of the conductor and  $l$  is the distance from the axis of rotation. The field predicted in equation (2) has in fact been observed, with the correct order of magnitude, in an experiment by Beams<sup>4</sup>. This experimental

verification is central to our discussion, and we shall henceforth consider equations (1) and (2) as firmly established.

Using a few basic ideas of the theory of general relativity, we can show that a force analogous to a centrifugal force acts on the ions and electrons in a conductor in a gravitational radiation field. Consider a one-dimensional system along the  $x$  axis. The metric for plane wave gravitational radiation moving in the  $z$  direction<sup>4,5</sup> is

$$ds^2 = dt^2 - (1 + h(t-z))dx^2 \quad (3)$$

(We use units in which  $c = 1$ , and assume the radiation has '+' linear polarisation.) The radiation field,  $h$ , is dimensionless and constitutes a very small perturbation on the flat-space metric. In this metric field, a freely-falling test particle experiences no coordinate acceleration, and thus two test particles at rest and separated by coordinate distance  $dx$  will remain so<sup>6</sup>. On the other hand the measurable physical separation, given by<sup>5,8</sup>

$$l = (1+h)^{1/2}dx \simeq (1+h/2)dx \quad (4)$$

will change as a function of time, and the two test particles will experience a relative acceleration. On this basis, we may associate a Newtonian tidal force with the radiation field, equal to the mass of a test particle times its acceleration referred to a fiducial test particle. From equation (4) this force is given by

$$F = m\ddot{h}dx/2 \simeq m\ddot{h}l/2; \ddot{h} \equiv d^2h/dt^2 \quad (5)$$

It is clear that this result must be general, and the tidal force will act on all particles, freely falling or not.

To visualise the effect of this gravitational tidal force on the ions and electrons in a conductor, it is convenient to consider a conductor whose centre ( $l = 0$ ) is in free fall. Then a force  $(\frac{1}{2}m\ddot{h})l$  will act on an ion at distance  $l$  from the centre, and in complete analogy with equation (2), an electric field  $E$  and associated voltage  $V$  must result

$$E = \Lambda m\ddot{h}l/2e; V = \Lambda m\ddot{h}l^2/4e \quad (6)$$

We must emphasise the theoretical foundation of this result. It rests entirely on equation (2), which we consider to be experimentally established, and on equation (5). If gravitational radiation exists, it is hard on dimensional grounds alone to conceive of a gravitational theory in which an equation analogous to (5) does not hold. Equation (6) would thus seem to rest on equally firm ground.

We can estimate the voltage  $V$  in equation (6) for some radiation sources studied by ref. 9. For the Crab pulsar a very rough estimate is  $h \sim 10^{-27}$  at a frequency  $\omega = 2\pi(60) \text{ Hz} = 377 \text{ Hz}$ ; then with  $m$  taken as the mass of a copper ion and  $l$  as 2 km we obtain a very small value of  $\sim 10^{-23} \text{ V}$ . For a typical supernova in the Virgo cluster of galaxies  $h \sim 10^{-21}$  at a characteristic frequency of  $\omega \sim 6 \text{ kHz}$ ; with  $\ddot{h} \sim h\omega^2$  we then obtain a more optimistic value of  $\sim 10^{-15} \text{ V}$ . Such supernova occur about once a month. Supernovae in our Galaxy would give a voltage  $\sim 3$  orders of magnitude greater, but are of less interest since they occur about once a century.

It is amusing to speculate very briefly on the qualitative design of a practical antenna based on these ideas. To obtain the voltage differences calculated above over a small distance we might consider two conductors of different metals many kilometres in length, the voltage difference at the ends then being proportional to the difference in the values of  $\Lambda$  for the two metals. It would presumably be preferable to use superconductors to minimise thermal and other spurious noise, and a superconducting cover has the added advantage of being a perfect shield against external electromagnetic radiation.

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## Palaeozoic and Mesozoic age trends for some ring complexes in Niger and Nigeria

OVER 40 individual granitoid ring complexes exhibiting similar petrological and geochemical variations have been recognised in Nigeria<sup>1</sup>. They range in size from 1,500 km<sup>2</sup> to < 2 km<sup>2</sup>. The ring complexes form part of a more extensive chain along the ninth meridian, extending from the north of the Air Massif through southern Niger and northern Nigeria to the north-western margin of the Benue Trough in Nigeria (Fig. 1). In

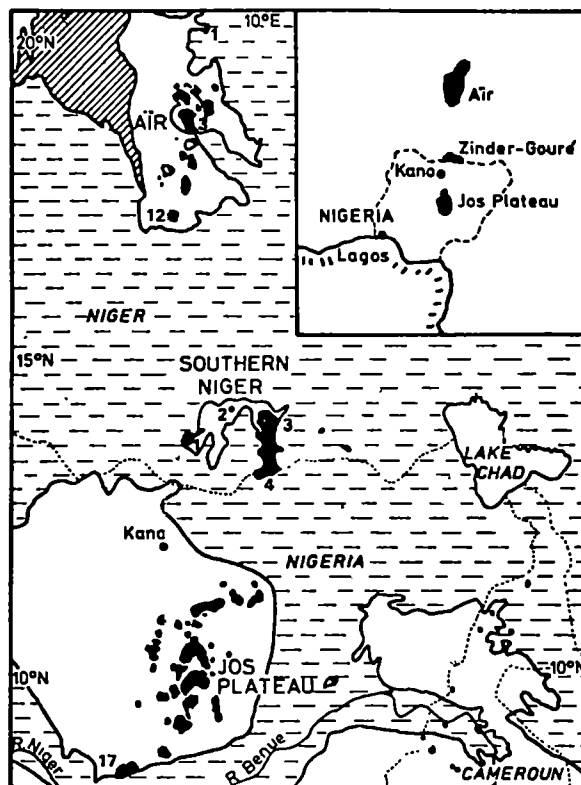


Fig. 1 Distribution of the Palaeozoic and Mesozoic ring complexes in Niger and Nigeria. The identification numbers of individual complexes are taken from ref. 1: 1 Air (mid-Palaeozoic); 1 Adrar Bous, 3 Tamgak-Enfoud group, 12 Tarraouadi; southern Niger (Upper Palaeozoic); 1 Zinder, 2 Tchouli-Zarniski, 3 Gouré, 4 Matsena; Nigeria (Mesozoic), 17 Afu. Shading: clear, Precambrian 'Basement'; diagonal, Palaeozoic; horizontal, Cretaceous-Recent.

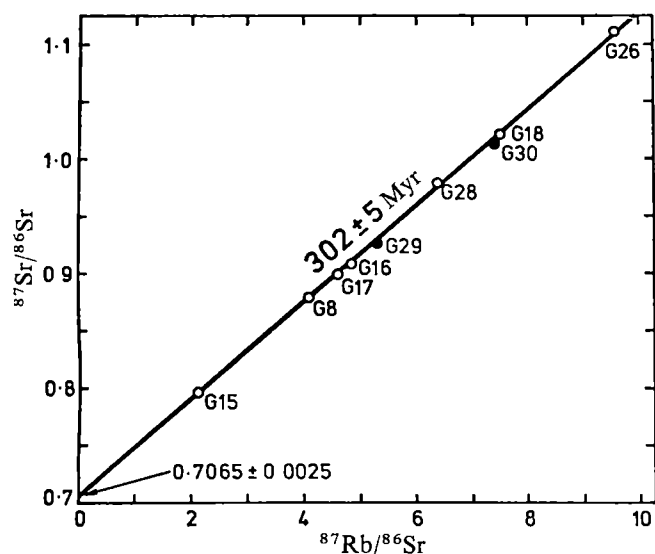


Fig. 2 Rb-Sr whole-rock isochron for the Gouré complex, Mounio Massif, southern Niger. Open circles with G numbers 8–28 represent samples from Gouré. Two additional samples G29 and G30 shown by closed circles, were collected from Adoumchi 20 km south of Gouré airport.  $\lambda = 1.39 \times 10^{-11} \text{ yr}^{-1}$ .

general the suite of Nigerian complexes are concentrated in a 200-km wide north-south zone and centred around the Jos Plateau. We have previously<sup>2</sup> investigated the group of ring complexes forming the central zone of anorogenic magmatism in Nigeria, and found a southerly decreasing age trend from early Jurassic to mid-Jurassic times. We give here further evidence for this trend, and point out that its recognition may be useful in ascertaining the drifting motion of the African continent.

The radiometric evidence<sup>2</sup> we found was consistent with cross-cutting relationships of individual complexes, but the validity of any systematic age sequence in chains of igneous ring complexes has been questioned<sup>3–5</sup>. We wish to draw attention to the following observations. New geochronological data for Zaranda, a syenite-related granitoid complex in northern Nigeria<sup>6</sup>, yields an age of  $190 \pm 10$  Myr. In contrast the Afu complex, which represents the southernmost Nigerian ring structure exposed beneath the Cretaceous–Recent sedimentary cover, has yielded a mean age of  $144 \pm 2$  Myr. This range of dates indicates that the individual granitoid ring complexes were not emplaced into the upper crust at the same time (150 Myr

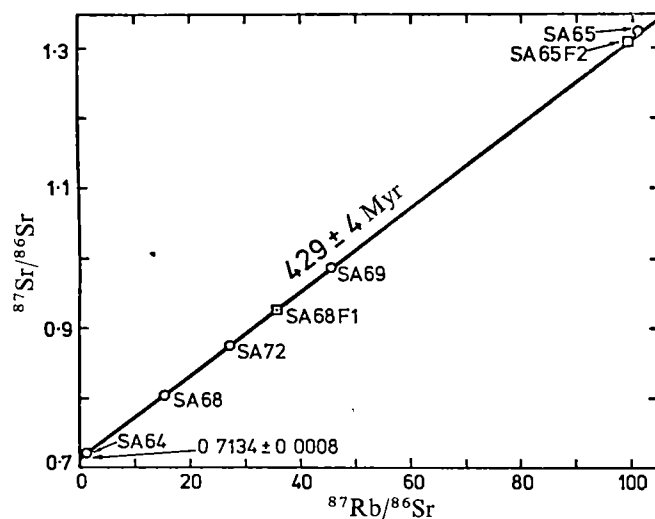
ago) but evolved during the Upper Triassic and Jurassic. Many of the complexes display a complete magmatic cycle, and evidence suggests that the development of an individual centre was established  $\leq 5$  Myr and then migration continued.

There are two anorogenic ring complex sub-Provinces in Niger represented geographically by southern Niger and by the Air Massif. The southern Niger region<sup>1</sup>, consists of the complexes of Zinder, Tchoumi-Zarniski and the southerly trending Mounio Massif from Gouré to Matsena (Fig. 1), a series of overlapping centres similar to the Sara-Fier Complex in Nigeria<sup>7</sup>, together with several isolated centres further to the east. Seven subvolcanic granites and porphyries from the Gouré Complex plot on a concordant isochron (Fig. 2) corresponding to an age of  $302 \pm 5$  Myr. Two samples from Adoumchi, the subsequent centre south of Gouré, plot slightly below the isochron suggesting a younger age of  $\sim 295$  Myr. The granite at Zinder yields an age of  $330 \pm 6$  Myr. The age-range for the southern Niger region remains to be confirmed in detail, but it is reasonable to conclude that anorogenic magmatism took place between 340 and 290 Myr ago, and that this period should be regarded as chronologically different from the younger period of Nigerian anorogenic magmatism. On the chronostratigraphic time scale<sup>8</sup>, the ring complexes of southern Niger are Carboniferous in age, and therefore in spite of petrological and geochemical similarities, these structures are considerably older than the equivalent centres in Nigeria.

The ring complexes in the Air Massif<sup>1</sup> are generally more peralkaline than their counterparts in Nigeria, with sub-alkaline biotite granite absent or only a minor component in most of them. A further contrast is provided by the occurrence of massive coarse-grained anorthosite, in several complexes, and olivine gabbro, at Adrar Bous in the extreme north of Niger<sup>9</sup>. Six of the ring complexes lie in a southerly trending zone 250 km long, but there is a pronounced central concentration of overlapping complexes, the Tamgak-Enfoud Group (Fig. 1). Previously, the Air ring structures were believed to have been emplaced during the same interval as the 'Younger Granite' magmatism in Nigeria, although subsequent estimates based on common lead<sup>9</sup> and K–Ar (ref. 10) methods gave inconsistent dates between 200 and 300 Myr. Preliminary new Rb–Sr whole rock and mineral age determinations for Adrar Bous and Enfoud give dates of  $429 \pm 4$  Myr and  $430 \pm 15$  Myr respectively. The concordant isochron for Adrar Bous is shown in Fig. 3. If this age is accurate for the granites at Adrar Bous, it means that anorogenic magmatism commenced in the Air region during early Silurian times. Stratigraphic evidence at Adrar Bous does not support that, however: the ring complex intrudes and metamorphoses sedimentary rocks which have been assigned a Lower Devonian age<sup>9</sup>. Nonetheless, if our age data are accepted, it means that the anorogenic granites in the north and central areas of the Air Massif are mid-Palaeozoic in age and are chronologically different from their counterparts in southern Niger and Nigeria. An unpublished age ( $\sim 380$  Myr) for Tarraouadji, the southernmost complex in the Air Massif (G. Marinelli, R. Black, personal communication) suggests that the Air ring complexes evolved during the Silurian and Devonian periods.

Accepting the concept of sublithospheric heat sources for mid-plate magmatism<sup>11</sup>, the chains of ring structures in the Niger–Nigerian Province may be useful in charting the continental drift of Gondwanaland before fragmentation. According to Briden<sup>12</sup>, there were quasistatic intervals during the Palaeozoic and Mesozoic eras when the Gondwana palaeomagnetic pole positions were stationary for varying lengths of time. Although there is some disagreement regarding the exact chronostratigraphic timing of the palaeomagnetic pole groupings in the Upper Palaeozoic, an earlier Middle-Palaeozoic quasistatic interval is recorded<sup>13</sup> which coincides with the evolution of the ring complexes in Air. The systematic age range for the Nigerian ring complexes also falls within a later Mesozoic quasistatic interval. Most workers accept that actual polar wandering does not normally occur, but that the continents

Fig. 3 Rb-Sr whole-rock and mineral isochron for the Adrar Bous complex, Air Massif, northern Niger. O, Whole rocks (SA) □, alkali feldspar separates (F1 and F2).  $\lambda = 1.39 \times 10^{-11} \text{ yr}^{-1}$ .



themselves have drifted rapidly during palaeomagnetic pole shifts and have remained either stationary or moved slowly during quasistatic intervals. It is perhaps significant that in the Niger-Nigerian Province there exists three groups of granitoid ring complexes which could, with more geochronological evidence and palaeomagnetic measurements, provide a complete record of the movement of the African continent from the beginning of the Silurian to the end of the Jurassic. The age trends of the ring complexes represent the intervals during the Palaeozoic and Mesozoic eras when Africa moved slowly; the absence of intervening ring structures indicating that Africa drifted rapidly.

The long time span from Adrar Bous (429 Myr) to Afu (144 Myr) compared with their small degree of latitude separation is, however, at variance with the palaeomagnetically predicted amount of drift over a single fixed point heat source.

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## Age of the migmatisation in the Dalradian of Shetland

WE have dated samples of schistose granite from Shetland using Rb-Sr methods. This provides further evidence for the early phase of Caledonian high grade metamorphism and migmatisation (the Grampian event) which has been detected all the way from western Ireland to northern Norway.

The Colla Firth Group, part of the East Mainland Succession of Shetland, is predominantly composed of steep to vertically-dipping regionally metamorphosed felspathic sandstones with a thickness of up to 4 km (refs 1, 2). These rocks have been correlated with the middle Dalradian of Scotland<sup>2,3</sup>. They contain a conformable belt of gneiss 1-2 km wide called the Colla Firth permeation belt<sup>1</sup> which can be followed from the southern end of Shetland for ~ 50 km to the north where it is intersected by the Nesting fault and offset 16 km to the south. The offset part of the belt can be followed for a further 20 km to the north-east before it disappears into the sea (Fig. 1). K-Ar ages of ~ 420 Myr have been obtained from nearby rocks<sup>4</sup>.

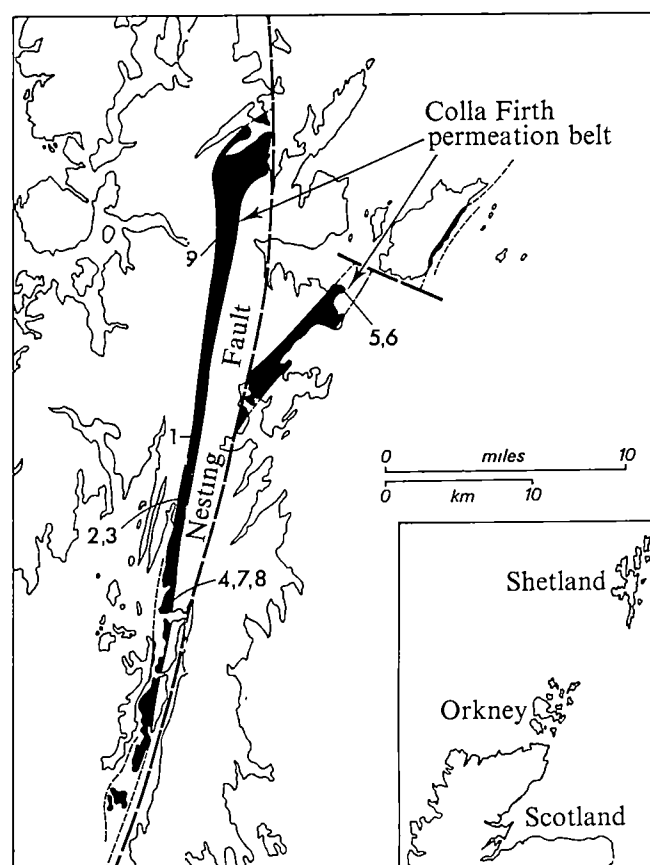


Fig. 1 Location of specimens.

Along most of the length of the belt the regionally metamorphosed rocks have been changed from semipelites and psammites to gneisses, characterised by streaks of coarse quartz and felspar, and to rather homogeneous granitoid gneisses. Along its entire exposed length the belt contains frequent intrusive sheets of non-schistose pegmatite and of schistose granite. In places it contains much larger masses of the schistose granite a kilometre or more across. All of these intrude both the gneisses and the adjacent unmigmatized rocks. It has been argued elsewhere that the migmatite belt was formed after the tectonising regional metamorphism of the adjacent rocks, this latter metamorphism being the earliest one discernable in the Colla Firth Group<sup>2,3</sup>. According to this interpretation, the schistosity in the granite veins and the tectonite fabric of the gneisses are the result of late movements accompanying the migmatisation. May<sup>5</sup> has claimed, however, that the schistosity in the granite veins and the schistosity in the regional metamorphic rocks are the same, so that the migmatisation and the regional metamorphism must have occurred at the same time.

To determine the age of migmatisation, eight whole rocks samples of schistose granite were analysed for Rb and Sr.

Table 1 Analytical data

Sample number	Rb(p.p.m.)	Sr(p.p.m.)	<sup>87</sup> Rb/ <sup>86</sup> Sr	<sup>87</sup> Sr/ <sup>86</sup> Sr
1	219.3	147.4	4.31	0.7540
2	238.9	135.4	5.22	0.7597
3	187.8	164.0	3.41	0.7460
4	190.0	164.9	3.43	0.7416
5	102.1	243.9	1.21	0.7313
6	162.0	193.1	2.43	0.7402
7	209.9	133.8	4.54	0.7555
8	248.6	54.99	13.24	0.8206
9	171.9	172.1	2.90	0.7376



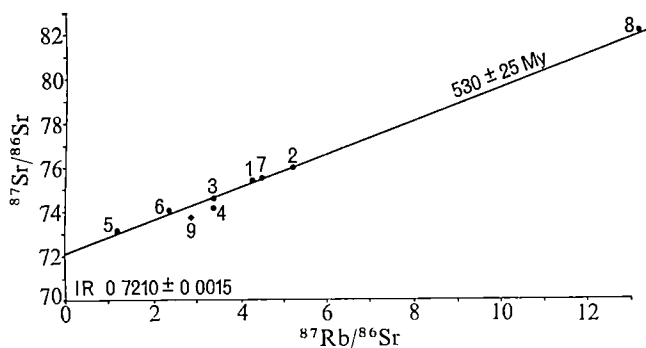


Fig. 2 Rb/Sr whole-rock isochron.

They are all composed of quartz, muscovite, plagioclase, microcline and biotite. Specimen 9 is from a granite gneiss intermediate in nature to the schistose granites and the migmatite gneisses. It was included to determine whether it could be correctly classed as a schistose granite.

Following preliminary analyses by X-ray fluorescence to determine Rb and Sr contents for sample selections and for spiking purposes all samples were analysed by standard isotope dilution techniques and the results of these analyses are given in Table 1 and plotted in Fig. 2. Sample 9 is not included in the isochron calculation which, using York's method II<sup>6</sup>, yields an age of  $530 \pm 25$  Myr with an initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of  $0.7210 \pm 0.0015$ . This age and all other Rb-Sr ages quoted are calculated using  $\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$ ; the errors given are all  $2\sigma$ . Considering the distribution of the data points, it is possible that sample 4 is associated with 9, and that 8, because of its relatively high Rb/Sr ratio, has a different source. As no geological or petrographical evidence could be found to support these possibilities they have not, however, been excluded. In fact, the ages obtained by excluding various selections of these data points from the isochron calculation all fall within the lower uncertainty limit of the preferred value given above.

The initial  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio of 0.7210 is in accordance with a crustal melting source for the schistose granite veins and thus with their derivation from the gneisses of the permeation belt. The age of  $530 \pm 25$  Myr for the schistose granite veins is thus believed to be a good estimate of the age of the permeation belt as a whole. If May<sup>5</sup> is correct this is also the age of the main phase of the regional metamorphism, but it is more likely that this took place earlier.

This age of  $530 \pm 25$  Myr for migmatization in Shetland is in complete agreement with ages determined for this event in Connemara,  $520 \pm 30$  Myr (refs 7, 8) and North Norway,  $530 \pm 35$  Myr (refs 7, 9). In Scotland, Bell's<sup>10</sup> determination of the age of the Ben Vuirich granite has been recalculated by Dunning<sup>7</sup> to be 511 Myr. This granite is about the same age as adjacent migmatites in middle Dalradian rocks, the Duchray Hill Gneiss, according to H. J. Bradbury (personal communication). The age of the schistose granite from Shetland agrees with ages for the main phase of high grade metamorphism in the other parts of the Caledonian fold belt.

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## Simultaneous measurements of $\text{H}_2\text{O}$ , $\text{NO}_2$ and $\text{HNO}_3$ in the daytime stratosphere from 15 to 35 km

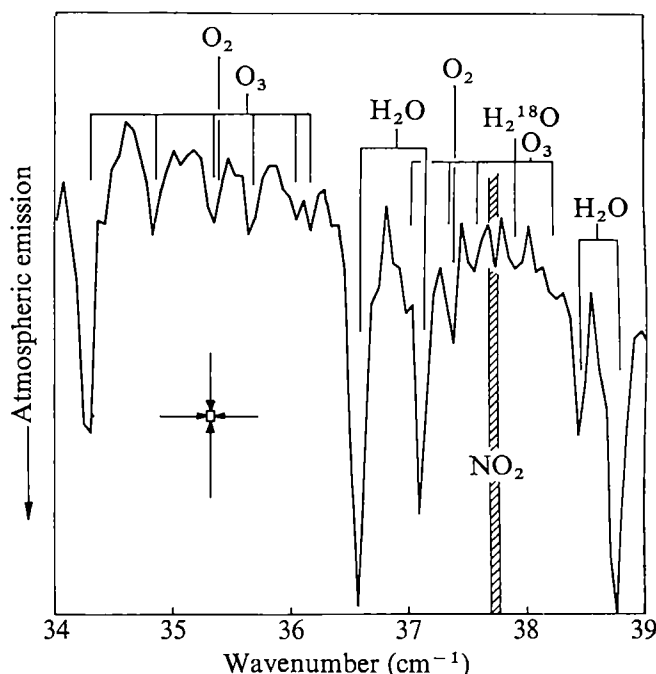
USING Fourier transform spectroscopy in the far infrared with a Michelson interferometer, and helium-cooled bolometric detectors, it is possible to study the emission spectrum of the stratosphere at high resolution ( $0.05 \text{ cm}^{-1}$ ) in the spectral range  $5-45 \text{ cm}^{-1}$  (see refs 1-3).

We report here initial results from two daytime high altitude balloon flights undertaken during the past year, using far infrared Michelson interferometers, to measure concentrations of several trace gases to altitudes of about 35 km. The aim of the work was to investigate the concentrations of several gases simultaneously, using the wide bandwidth and high resolution capabilities of the Fourier technique. It is possible to obtain information on  $\text{H}_2\text{O}$ ,  $\text{O}_3$ ,  $\text{HNO}_3$ ,  $\text{N}_2\text{O}$  and  $\text{NO}_2$  in one scan of the far infrared interferometer. Results on  $\text{H}_2\text{O}$ ,  $\text{HNO}_3$  and  $\text{NO}_2$  only are presented here, measured at 1200 LT.

The flights were made from the Centre National d'Etudes Spatiales (CNES) balloon launching facility at Aire-sur-l'Adour in south-western France, on September 12 and September 20, 1974. Launches were achieved at about 0900 LT, and observations at altitude (34-36 km) were made during the local noon period.

The experimental system was similar to that used in previous aircraft experiments, though with several modifications and improvements to accommodate the more

Fig. 1 Portion of a far infrared emission spectrum obtained while floating at 35 km, at a zenith angle of  $92^\circ$ . The curve is an average of five runs each taking 8 min to record. The spectral resolution is  $0.07 \text{ cm}^{-1}$  (unapodised). Shaded line, range of possible centre wavenumbers for the  $\text{NO}_2$  Q branch, as judged from theoretical and experimental sources, arrows, resolution width and signal-to-noise ratio (estimated from reproducibility of different spectra).



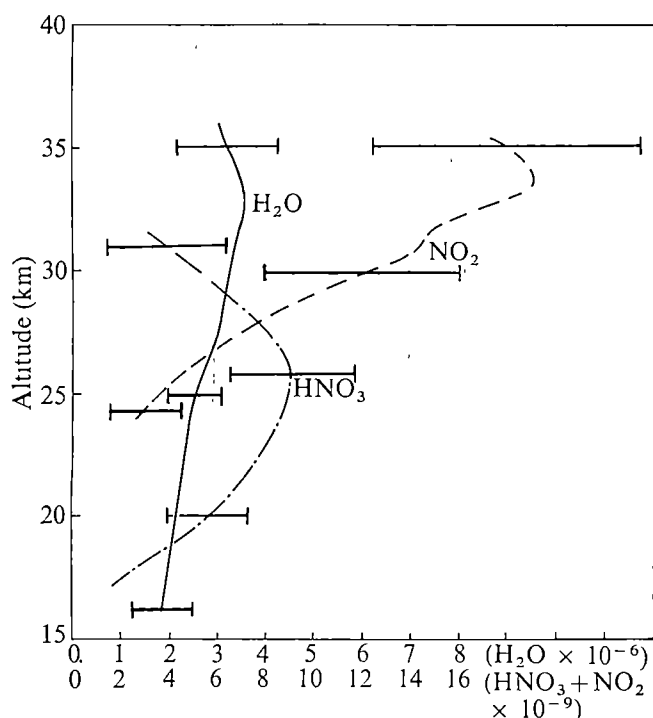


Fig. 2 Vertical profile of mixing ratio for  $\text{H}_2\text{O}$ ,  $\text{HNO}_3$  and  $\text{NO}_2$ , measured during the flight of September 20, 1974 at  $44^\circ\text{N}$   $0^\circ\text{W}$ . Uncertainties given in the text. Mass units are given for  $\text{H}_2\text{O}$  and volume units for  $\text{NO}_2$  and  $\text{HNO}_3$ .

difficult and remote operating conditions encountered on a balloon flight. A full description will be given elsewhere, but it should be noted that limb scanning was used to build up a vertical profile of concentration while the package floated at ceiling. The instrument was stabilised against motions of the gondola by reference to the solar elevation angle which was measured in real time; these motions proved to be very small (amplitude less than  $0.1^\circ$ ). A helium-cooled InSb electron bolometer was used.

Figure 1 shows a portion of a typical spectrum, for the range  $34\text{--}39\text{ cm}^{-1}$ , at a spectral resolution of  $0.07\text{ cm}^{-1}$ . Features seen are attributable to  $\text{H}_2\text{O}$ ,  $\text{O}_2$ ,  $\text{O}_3$  and  $\text{NO}_2$  (refs 2–6); other features attributable to these gases, and to  $\text{HNO}_3$  and  $\text{N}_2\text{O}$ , occur at lower wavenumbers.

The results of the September 1974 flights for  $\text{H}_2\text{O}$ ,  $\text{HNO}_3$  and  $\text{NO}_2$  between 15 and 35 km are shown in Fig. 2. It is an important feature of our technique that the profiles were measured simultaneously; the measurements were made during the period  $\pm 1\text{ h}$  around noon on September 20, 1974. The uncertainties are typically  $\pm 15\%$  for  $\text{H}_2\text{O}$ ,  $\pm 25\%$  for  $\text{HNO}_3$ , and  $\pm 35\%$  for  $\text{NO}_2$ , and are attributable to uncertainties in radiometric calibration, line intensity measurement, observation angle, and spectroscopic parameters such as integrated line strength.

The results for  $\text{H}_2\text{O}$  show a gradual increase in volume mixing ratio from about 3.0 p.p.m. (by volume) at 15 km to about 5.5 p.p.m. at 33 km. For  $\text{NO}_2$  we found about 4 parts per  $10^9$  at 25 km rising to about 18 parts per  $10^9$  at 33 km, with slight evidence of a maximum at this level. In the case of  $\text{HNO}_3$  a pronounced maximum occurs at about 25 km with mixing ratios of about 9 parts per  $10^9$ .

The water vapour result agrees well with previous measurements made from aircraft<sup>2,3</sup> and with data obtained from an earlier single balloon flight over the UK<sup>7</sup>. We have not previously observed such a marked increase of mixing ratio with altitude as is shown in Fig. 2, however, though several measurements support the occurrence of somewhat more humid layers of air above 30 km (refs 8, 9 and 16) in some cases with mixing ratios up to 10–20 p.p.m. There remains a considerable degree of

variability between results above about 30 km, however, and it must be accepted that our understanding of the humidity of the upper stratosphere is far from complete. It should be noted that the apparent increase with height observed in the present results is significant in terms of the relative uncertainty attributable to random errors at different altitudes, since most of the uncertainty indicated in Fig. 2 arises from systematic effects.

In the case of nitric acid, our results agree well with those of Murcray *et al.*<sup>10</sup>, who at mid-latitudes have reported volume mixing ratios around 5 parts per  $10^9$  volume at about 23 km. The extensive data reported by Lazrus *et al.*<sup>11</sup> seem to be consistently lower in absolute value than our own results and those of Murcray, however. A nitric acid profile suggested by Fontanella *et al.*<sup>12</sup>, for a similar latitude to that at which our measurements were taken shows a lower peak (nearer 20 km) but absolute values of mixing ratio similar to our own.

Turning to nitrogen dioxide, there have been a large number of measurements in the past two years of this species, with some disparity between the different results. Murcray *et al.*<sup>10</sup> report values ranging from 1.5 parts per  $10^9$  at 20 km to 5 parts per  $10^9$  at 28 km. Fontanella *et al.*<sup>12</sup> report values of about 1 part per  $10^9$  at 20 km and 0.3 parts per  $10^9$  at 15 km. The last two sets of data refer to sunrise or sunset conditions since the solar absorption/limb-sounding method has been used in each case, and the exact timing of the measurements relative to local sunrise/sunset is not available. It is important to identify this relative time very accurately since it is now known that the concentration of  $\text{NO}_2$  varies rapidly at dawn and sunset (some recent data have been obtained on the variability of  $\text{NO}_2$  at sunrise from our own measurements, and these will be published shortly). Farmer *et al.*<sup>13</sup> have also reported  $\text{NO}_2$  measurements using the solar absorption technique, and obtained about 1.8 parts per  $10^9$  between 15 and 20 km.

Daytime, as well as early morning and evening measurements of  $\text{NO}_2$  have been reported by Brewer *et al.*<sup>14</sup>. They report values of around 50 parts per  $10^9$  at 30 km at mid-day, but Johnson<sup>15</sup> has reinterpreted the data and has obtained values nearer 10 parts per  $10^9$ , with only slightly lower values at dawn. Results obtained by Chaloner *et al.*<sup>16</sup> show midday profiles ranging from about 6 parts per  $10^9$  at 20 km to 15 parts per  $10^9$  at 35 km.

Clearly, therefore, there is a range of results; at 25 km for example, values vary from about 1 to 10 parts per  $10^9$ . Our own value here is  $\sim 3.5$  parts per  $10^9$ . In measurements as difficult as this, however, it is in our view reassuring that such close agreement is obtained, and, certainly natural variations must be allowed for in assessing the various results.

Further balloon flights have been made, in April 1975, which spanned sunrise. The results of these flights will be reported shortly, and contain information on the diurnal variability of  $\text{NO}_2$ .

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## Search for correlation between geomagnetic disturbances and mortality

It is generally accepted that the Sun affects the Earth's magnetosphere and ionosphere by variations in the ultraviolet and X-ray radiation and in the solar wind due both to short lived disturbances such as flares<sup>1</sup> and to long lived effects such as that associated with the 'sector structure' of the interplanetary magnetic field<sup>2</sup>. There have, however, been repeated claims<sup>3,4</sup> for well over a century that the Sun influences many other processes on Earth, including terrestrial weather and human disorders. Friedman, Becker and Bachman<sup>5-7</sup> have presented evidence for an association between geomagnetic storms, cosmic-ray flux variations and psychological behaviour. The term geomagnetic storm is used for worldwide fluctuations in the Earth's field with a scale of about 100γ over a period of several hours, caused by the impact of a solar plasma front on the magnetosphere. A number of Russian scientists<sup>8-10</sup> have claimed that there is a real association between geomagnetic storms and the incidence of various human diseases. Within this general area, one of the most active areas of current research seems to be the correlation of solar activity and myocardial infarction and stroke. We have searched for a similar correlation in the USA but have failed to find one.

Gnevyshev and Novikova<sup>10</sup> refer to the work of several other groups, published in Russian and unavailable to us. Seventeen papers are referenced, all of which are said to suggest the possibility of direct effects of solar activity on living organisms. They present evidence of two types: first, the results of direct experiments in which biological systems were placed in artificially induced electromagnetic oscillations and second, studies of correlation between geomagnetic indices and mortality due to heart disease and stroke, using the method of superposed epochs and comparison of averaged medical data on days of different geomagnetic activity. The results presented seem to indicate positive correlations, but no estimates of statistical significance are given. We therefore performed similar statistical analyses using US data. Daily numbers of deaths due to specific causes were obtained from the National Center of Health, referring to the actual date of death rather than the registration date.

Geomagnetic activity is measured by magnetic indices. Local *K* indices are based on the measured amplitude of variation over a 3-h interval; corrections for local effects are applied to give world *Ap* indices. Another form of geomagnetic activity is due to hydromagnetic emissions which are enhanced after geomagnetic storms and produce ultra low frequency (~ 1 Hz) fluctuations in the terrestrial magnetic field. This type of activity is measured by a local *W* index which represents the number of 15-min intervals in a 24-h day that contain hydromagnetic emissions.

We compared daily numbers of deaths in the USA due to coronary heart disease and stroke for 1962-66 with the corresponding *Ap* indices using three methods. Mortality data were normalised to remove weekly and seasonal variations and a long term secular trend, since we were looking for short term correlations with natural events. This was done by renormalising with respect to average yearly and weekly curves for the 6-yr period and a linear ramp obtained by least-squares fitting. Mortality and geomagnetic data are shown in Fig. 1.

Our first method was to calculate the correlation coefficient  $r_k$  between daily deaths  $D_i$  and  $A_i$ , the daily value of *Ap*, where  $i (= 1, \dots, N)$  enumerates days. In the formula

$$r_k = \frac{\sum_{i=1}^{N-k} (A_i - \bar{A})(D_{i+k} - \bar{D})}{(N-k)\sigma_A\sigma_D} \quad (1)$$

$k (>0)$  represents the number of days *D* lags *A*;  $k < 0$  indicates a lead;  $\bar{A}$ ,  $\bar{D}$  are means of the time series;  $\sigma_A$ ,  $\sigma_D$  are the standard deviations. The standard error of  $r_k$  is given by

$$\sigma_{r,k} = (N - |k| - 1)^{-1/2}(1 - r_k^2) \quad (2)$$

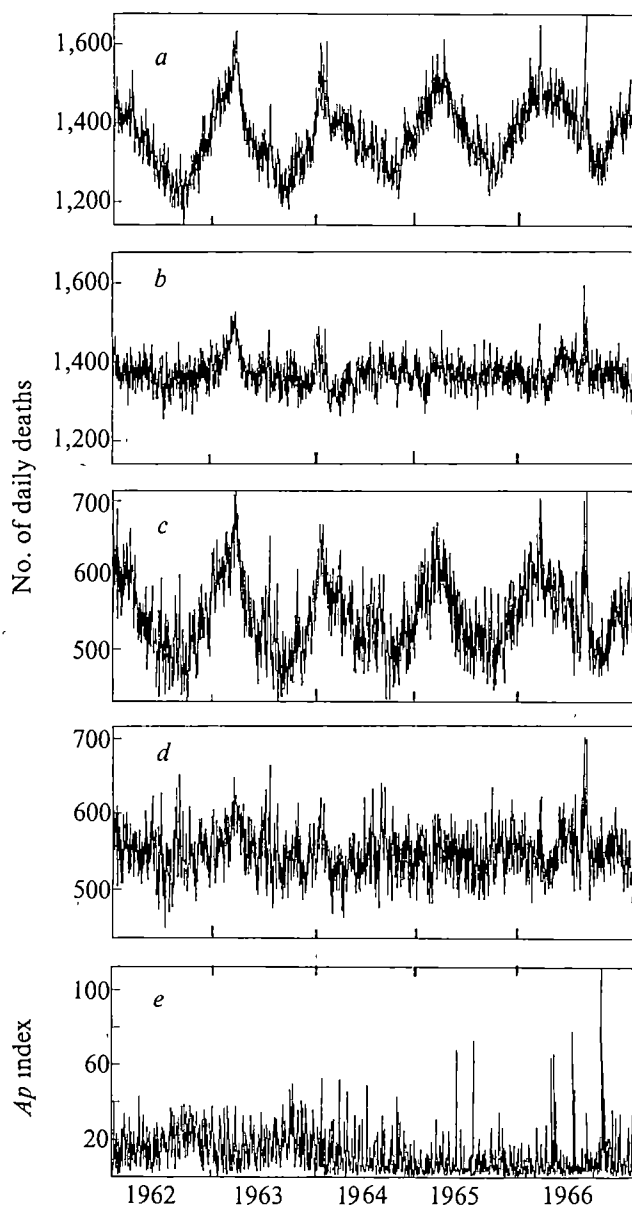
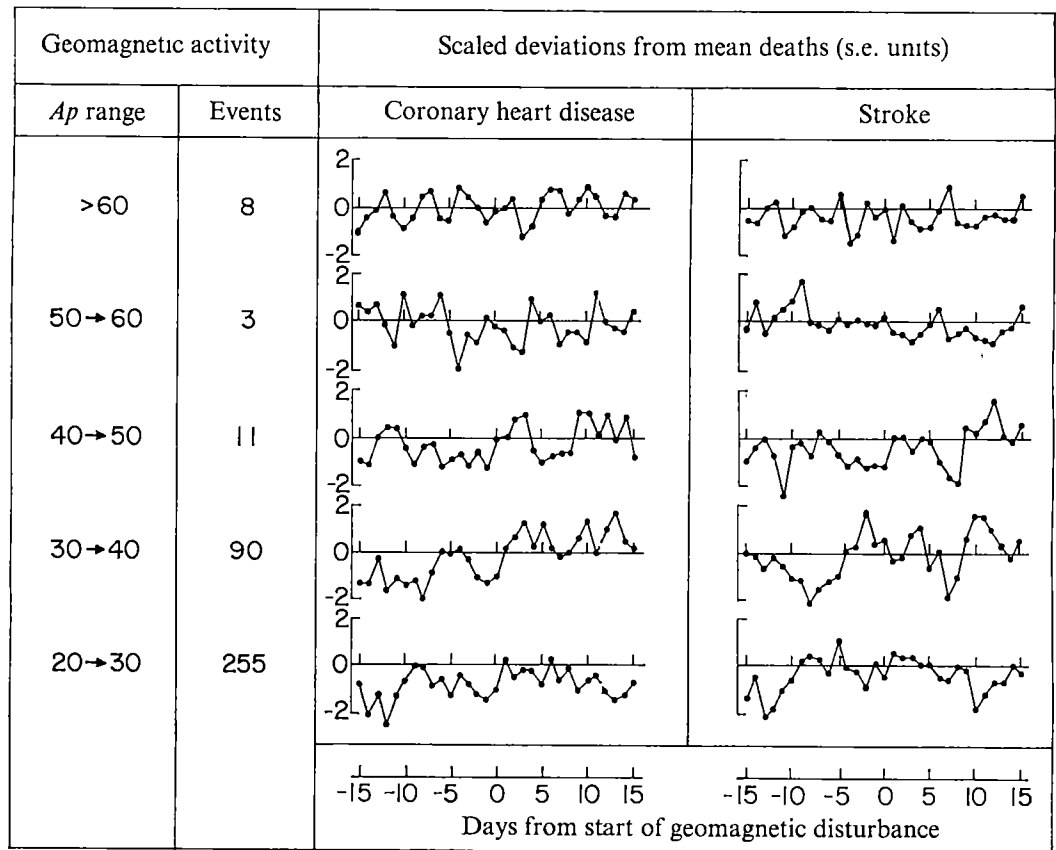


Fig. 1 *a*, Daily US mortality from coronary heart disease, 1962-66. Averaged deaths: Sunday, 1489.1; Monday, 1551.3; Tuesday, 1493.9; Wednesday, 1487.9; Thursday, 1489.6; Friday, 1504.9; Saturday, 1527.3. *b*, Normalised data obtained from *a*. Averaged deaths are the same for each day of the week and equal to 1507.1. *c*, Daily US mortality from stroke, 1962-66. Averaged deaths: Sunday, 549.3; Monday, 555.8; Tuesday, 547.8; Wednesday, 546.4; Thursday, 543.0; Friday, 547.1; Saturday, 552.3. *d*, Normalised data obtained from *c*. Averaged deaths are the same for each day of the week and equal to 549.1. *e*, Geomagnetic *Ap* index, 1962-66.

**Fig. 2** Superposed epoch diagrams for mortality due to coronary heart disease and stroke for the period 1962–66. An event occurs when  $A_p$  on day zero lies in the ranges given. Mean daily deaths for the total sample and standard deviations ( $\sigma$ ) about the mean: coronary heart disease 1507.1, 57.9, stroke 549.1, 27.3. Departures from the mean are measured in units of  $\sigma/\sqrt{n}$  where  $n$  is the number of events.



Results for  $-14 \leq k \leq 14$  are given in Table 1. We found no evidence for correlation at the  $3\sigma$  significance level, a criterion we used throughout the analysis. For nearly all  $k$ , the correlation coefficient is negative, indicating that a small, long term anticorrelation exists. This effect, which is not relevant to our study, arises from low frequency trends in the data.

As an alternative procedure, we constructed superposed epoch diagrams for deaths on days with  $A_p$  in selected ranges. Results are shown in Fig. 2, plotted as deviations from the mean daily deaths of the total sample. The deviations were scaled by  $\sigma/\sqrt{n}$  where  $n$  is the number of superposed events and  $\sigma$  is the standard deviation from the mean of the total sample, so that each plot would have the same width of scatter if the selected subsets were simply a random selection from the total sample. This allows direct comparison of the diagrams and easy detection of statistically significant deviations. In most of the diagrams, the averaged superposed daily deaths throughout the period are less than the total sample mean, another manifestation of the small long term trends already noted. No diagram gives evidence for a statistically significant association between daily deaths and magnetic index.

The third method of presentation was to plot averaged deaths as a function of magnetic index for  $-20 \leq k \leq 20$ . Again there was no evidence for a significant effect.

Since Gnevyshev and Novikova<sup>10</sup> studied the relationship between mortality levels and geomagnetic indices for individual cities, rather than an entire nation, the above analyses were repeated for 3 metropolitan areas. Daily deaths during 1964 and 1965 due to coronary heart disease in Phoenix, Honolulu and Washington, DC were compared with local  $K$  indices. Some results for zero lag are given in Fig. 3, using the third method of presentation. None of the results shows any evidence for correlation.

Finally, we compared daily deaths due to coronary heart disease in San Francisco in 1964 and 1965 with the  $W$  index for micropulsations. We did this test because Gnevyshev and Novikova attribute their claimed relationship between geomagnetic activity and mortality levels to low frequency fluctuations in the geomagnetic field. Some results for zero lag are given in

**Fig. 3.** These analyses also showed no evidence for an association.

In summary, our study does not support the findings of Gnevyshev and Novikova, nor their proposal for a new branch of science—heliobiology. It is possible that the correlations they find are either not statistically significant or not due to a causal relationship between geomagnetic disturbances and

**Table 1** Correlations between geomagnetic index  $A_p$  and daily US mortality from coronary heart disease and stroke, 1962–66

Lag index $k$	Correlation coefficients, $r_k$	
	Coronary heart disease	Stroke
-14	-0.061	-0.028
-13	-0.052	-0.037
-12	-0.067	-0.052
-11	-0.063	-0.069
-10	-0.041	-0.040
-9	-0.040	-0.017
-8	-0.030	-0.031
-7	-0.019	-0.039
-6	-0.034	-0.037
-5	-0.040	-0.012
-4	-0.033	-0.039
-3	-0.029	-0.024
-2	-0.052	-0.008
-1	-0.065	-0.021
0	-0.044	-0.018
1	-0.015	-0.037
2	-0.002	0.001
3	-0.010	-0.012
4	-0.025	-0.007
5	-0.023	-0.028
6	-0.014	-0.021
7	-0.028	-0.045
8	-0.019	-0.040
9	-0.009	-0.003
10	0.014	0.003
11	0.001	0.007
12	-0.005	0.004
13	-0.011	-0.007
14	-0.008	-0.012

Deaths lag behind geomagnetic event if  $k > 0$ , and lead if  $k < 0$ . Standard error in  $r_k$  is constant to 3 decimal places and equal to 0.023.



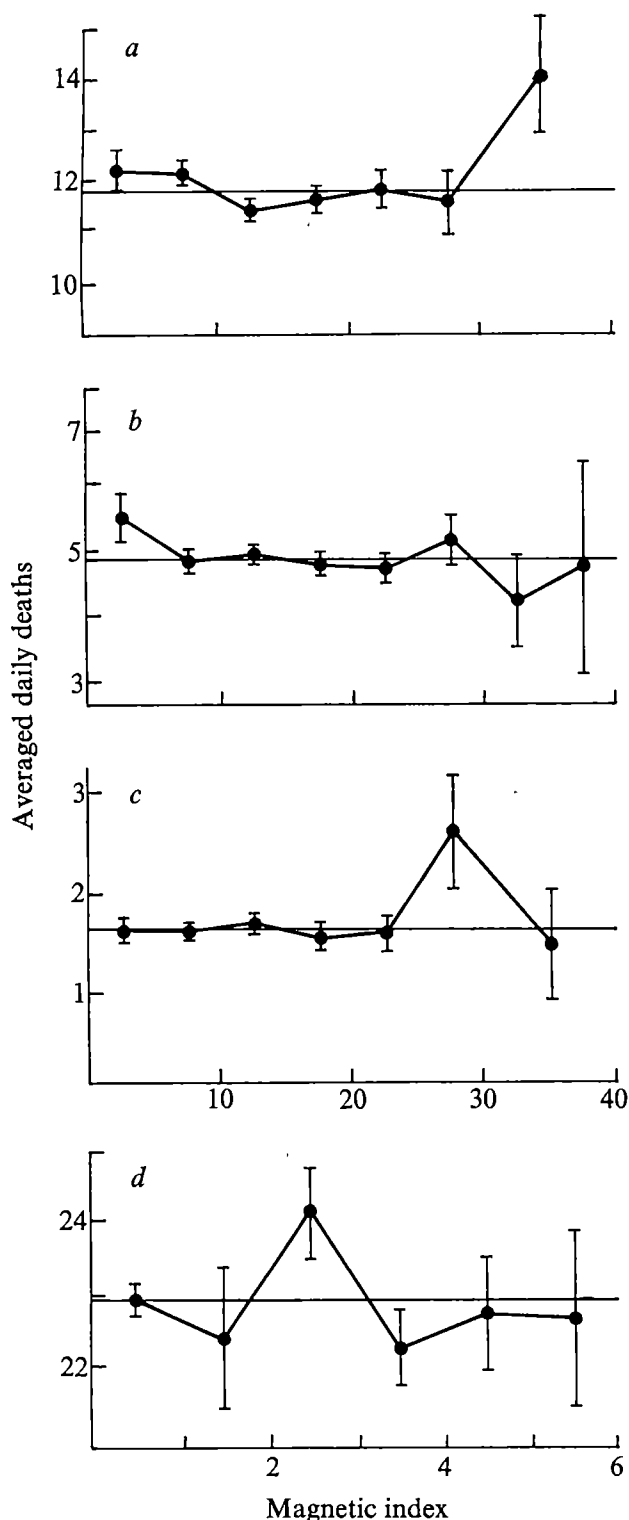


Fig. 3 Averaged mortality due to coronary heart disease as a function of magnetic index for zero lag. *a*, Washington, DC; *b*, Phoenix; *c*, Honolulu; *d*, San Francisco. For *a*, *b* and *c* the magnetic index used was the sum of local *K* indices for the day; *d* the local *W* index was used.

coronary heart disease and stroke. If their correlations are indeed indicative of a causal relationship, it will be necessary to determine whether it is sensitive to geographical location, to phase of the solar cycle or to some other parameter which might distinguish the samples we have analysed from those of the Soviet scientists.

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## Hardy-Weinberg ratios and rare male mating advantage

PETIT<sup>1</sup> and others have shown that in a variety of *Drosophila* species the mating success of male genotypes is often dependent on their frequency in the population. This frequency-dependent mating effect seems to be relatively unimportant in females, and by favouring rare male genotypes represents a possible mechanism by which genetic variation can be maintained in a population. Whether or not this form of selection is widespread in natural populations is unknown, although, in the laboratory at least, it has been shown that the phenomenon occurs in insects other than *Drosophila*<sup>2,3</sup>.

Can this type of selection be detected in the wild without resorting to mating experiments? Unfortunately precise mathematical analysis of the problem, even at the simplest level, is complex and requires the choice of specific functions to describe how male mating success changes with gene or genotype frequency<sup>4</sup>. A more general approach can, however, be adopted, which, while not providing exact answers, highlights two possible problems in detecting frequency-dependent mating effects in natural populations.

Consider a single autosomal locus segregating *n* alleles in a large population of some sexual species. Assume that the generations are separate and that all *n* alleles are maintained in the population by frequency-dependent male mating effects. Other selective forces are assumed to be equal over all alleles. Let the mating success of a male of genotype *ij* be denoted by the function *F<sub>ij</sub>* (which may be dependent on any number of the allele frequencies), the frequency of that genotype be *g<sub>ij</sub>* and the frequency of allele *i* be *p<sub>i</sub>*.

The frequency of each genotype in the next generation can now be calculated by considering the two populations of gamete types. The frequency of each allele in the pool of eggs is unaffected by selection but in the pool of sperm the frequency of allele *i* will be

$$p_i' = (g_{ii}F_{ii} + \sum_k g_{ik}F_{ik}/2)/\bar{w}$$

where *i* ≠ *k*, and  $\bar{w}$  ensures that the sum of all frequencies is one. The genotype frequencies in the next generation are given by

$$g_{ii}' = p_i p_i' \quad (1a)$$

$$g_{ij}' = p_i p_j' + p_i' p_j \quad (i \neq j) \quad (1b)$$

At equilibrium the new genotype frequencies are the same as the old and substituting (1a) and the equivalent relationship

for the homozygote  $j/j$  into (1b) yields the set of equilibrium equations

$$\hat{p}_i \hat{p}_j \hat{g}_{ij} = \hat{p}_i \hat{g}_{ji} + \hat{p}_j \hat{g}_{ii} \quad (2)$$

Given the formula relating an allele frequency to its constituent genotype frequencies and that both allele and genotype frequencies sum to one, then the equilibrium form (2) defines the Hardy-Weinberg ratios. Thus, in the conditions of this model, selective forces acting on the sexual success of only one sex cannot be measured, or even suspected, by looking for deviations from the Hardy-Weinberg ratios in a population at, or close to, genetic equilibrium.

A second approach, possible when females are fertilised by only one male per brood, is to assess the frequency of different matings. In particular, consider the mating of the two genotypes  $i/j$  and  $k/l$ . Provided that not all of  $i, j, k$  and  $l$  are the same, it is then possible to compare directly the frequency of such crosses when the female is  $i/j$  to when the male is  $i/j$ . The ratio of these two frequencies gives a direct estimate of the relative fitness of the two males ( $F_{kl}/F_{il}$ ).

The direct comparison of mating frequencies can be extended further in populations at genetic equilibrium, since genotype and allele frequencies can then be related by the Hardy-Weinberg result. For example, if the alleles,  $i, j, k$  and  $l$  are all different then the expected frequency of an  $i/j$  female mating with a  $k/l$  male is  $(2\hat{p}_i\hat{p}_j)(2\hat{p}_k\hat{p}_l)F_{kl}/\bar{w}$  and that for a  $k/j$  female mating with an  $i/l$  male is  $(2\hat{p}_k\hat{p}_j)(2\hat{p}_i\hat{p}_l)F_{il}/\bar{w}$ , thus the ratio of these two frequencies again gives a direct estimate of the relative fitness of the two males ( $F_{kl}/F_{il}$ ). The size and number of groups of matings which can be compared in this way depend on the number of alleles segregating. Table 1 shows examples of the largest groups of matings which can be compared directly for two, three and four or more alleles. Of course, all possible comparisons could be made, but this method has the advantage that expectations are independent of genotype and allele frequencies.

**Table 1** Examples of the largest number of direct mating frequency comparisons which can be made in an equilibrium population segregating 2, 3 or 4+ alleles

Number of alleles	Mating ♀	♂	Expected frequency (with selection)	Expected ratio (no selection)
2	1/2	1/2	$4p_1^2p_2^2F_{12}/\bar{w}$	4
	1/1	2/2	$p_1^2p_2^2F_{22}/\bar{w}$	1
	2/2	1/1	$p_1^2p_2^2F_{11}/\bar{w}$	1
3	1/2	2/3	$4p_1p_2^2p_3F_{23}/\bar{w}$	2
	2/3	1/2	$4p_1p_2^2p_3F_{12}/\bar{w}$	2
	1/3	2/2	$2p_1p_2^2p_3F_{22}/\bar{w}$	1
	2/2	1/3	$2p_1p_2^2p_3F_{13}/\bar{w}$	1
4+	1/2	3/4	$4p_1p_2p_3p_4F_{34}/\bar{w}$	1
	3/4	1/2	$4p_1p_2p_3p_4F_{12}/\bar{w}$	1
	1/3	2/4	$4p_1p_2p_3p_4F_{24}/\bar{w}$	1
	2/4	1/3	$4p_1p_2p_3p_4F_{13}/\bar{w}$	1
	1/4	2/3	$4p_1p_2p_3p_4F_{23}/\bar{w}$	1
	2/3	1/4	$4p_1p_2p_3p_4F_{14}/\bar{w}$	1

In the case of a sex-linked locus, the female population (assumed to be homogametic) is still in Hardy-Weinberg ratios at equilibrium (the males representing the allele frequencies directly). Therefore direct comparisons analogous to those of Table 1 can be made, although the maximum number of matings in each group of comparisons is reduced to two for a pair of alleles or to three otherwise (but note that the number of male genotypes is also reduced).

The use of direct mating comparisons in equilibrium populations can indicate and measure mating selection but the method has the disadvantage of not being powerful enough to dismiss

the possibility of even strong frequency-dependent effects. This is because under such circumstances selective values may be very similar at genetic equilibrium, and thus equilibrium analysis, however detailed, may be fruitless.

The preceding suggests that if frequency-dependent (or indeed constant) sexual selection is acting on one sex to maintain a polymorphism, then, at genetic equilibrium, deviations from Hardy-Weinberg ratios are not expected, but an analysis of the occurrence of specific matings may reveal the selection. Failure to demonstrate selection at equilibrium does not imply that it is not acting or that it is not important. Only the analysis of perturbation experiments can be used to eliminate this form of selection as an important stabilising influence.

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## Energetic cost of locomotion in Australian hopping mice

THE mode of locomotion of the hopping mouse can be compared with that of the kangaroo moving bipedally at speed. Because the latter cannot be monitored due to technical difficulties, I have used the hopping mouse in a study of the energetics of this type of locomotion at speeds of 0.5-7 km h<sup>-1</sup>. I found that at 2.5-3 km h<sup>-1</sup>, the stride frequency stabilised, as did steady-state oxygen consumption. Above 5 km h<sup>-1</sup> oxygen consumption again increased although stride frequency did not. The result of this pattern was that the costs of hopping in *Notomys cervinus* were markedly below those predicted for a quadruped. The overall pattern had similarities with those displayed by kangaroos<sup>1</sup>.

I used three specimens (34-38 g) of *N. cervinus*, the fawn hopping mouse, native to Australia, collected in stony desert in western Queensland. This species can be trained on a treadmill and hops readily. Energy cost was estimated from steady-state oxygen consumption measured for 15-30 min while the animals travelled at speeds of 0.5-7.0 km h<sup>-1</sup> inside a Perspex chamber (40×10×15 cm) with a flexible seal at its base. Air was drawn through the chamber at approximately 4 l min<sup>-1</sup>, and nitrogen, bled into the chamber at a constant rate, indicated that, at all speeds, there was no leakage of air out of the chamber. Oxygen consumption was measured with a Beckman F-3 paramagnetic analyser with a full scale of 20.5-21% oxygen. Air temperature was maintained at 26-28 °C so that shivering did not affect the oxygen consumption at lower speeds—such an effect was apparent in initial experiments at 17-19 °C. Strides were monitored using a video camera with a slow motion playback capability. Twenty sequences of twenty strides were counted for each animal at each speed, and stride length was calculated from stride frequency and treadmill speed.

Oxygen consumption was similar for all three animals, although one had a consistently higher consumption (Fig. 1). Between 0.5 and 2.5 km h<sup>-1</sup> oxygen consumption increased linearly with increasing speed. At these speeds the mice either 'trotted' with the hind legs moving independently or 'galloped' quadrupedally with the hind legs

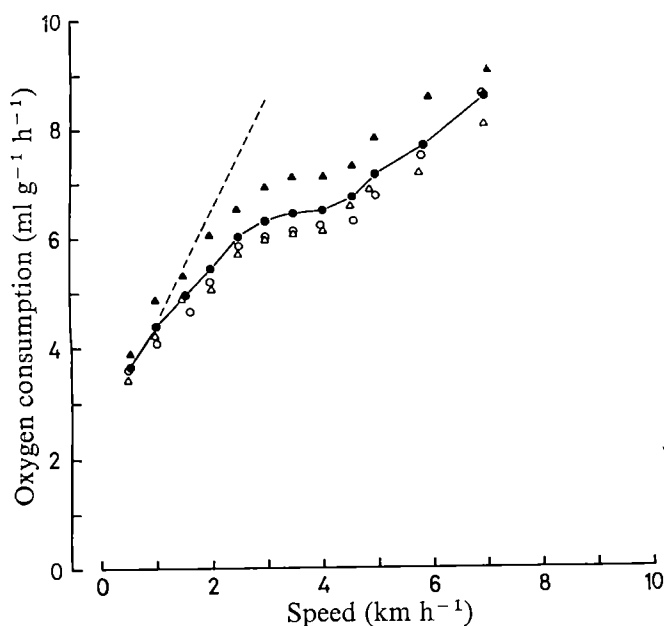


Fig. 1 Steady-state oxygen consumption of the hopping mouse *N. cervinus* as a function of speed. The mean values at each speed (●) are joined by the solid line. Values for individual animals are indicated by different symbols. The dashed line represents the oxygen consumption predicted for a mammal the same weight as the mean weight of the hopping mice, 36.6 g.

moving together. During this phase the energy cost did not diverge markedly from that predicted for a quadruped<sup>2</sup>. An increase in speed in this range was accomplished by increases in stride frequency and length (Fig. 2).

As in the case of kangaroos<sup>1</sup>, oxygen consumption reached a plateau at the speeds at which stride frequency became nearly constant, 3 km h<sup>-1</sup>. This stability in energy cost did not persist, however, although stride frequency remained constant at speeds > 7 km h<sup>-1</sup>. At > 4.5 km h<sup>-1</sup>, oxygen consumption again increased with an increase in

speed, in an apparently linear manner, but at a rate well below that predicted for quadrupeds (which is a behaviour we assume kangaroos would also show at their highest speeds).

One difference between the pattern of locomotion of hopping mice and of kangaroos was that there was no immediate shift to complete hopping at the speeds at which stride frequency and oxygen consumption tended to stabilise initially (3 km h<sup>-1</sup>). More or less continual bipedal hopping was not achieved by the mice until beyond 4.5 km h<sup>-1</sup>, and the animal with consistently high oxygen consumption did not achieve complete hopping, even at 7 km h<sup>-1</sup>. The overall picture for the relationship between type of gait and speed was similar for the two animals with the lower oxygen consumption: between 0.5 and 1.5 km h<sup>-1</sup> the mice mostly trotted, between 1.5 and 2.5 km h<sup>-1</sup> they mostly galloped, and above 2.5 km h<sup>-1</sup> some hopping was apparent, initially in the form of a sequence of four to six hops interspaced with galloping. At 3.0 km h<sup>-1</sup> galloping comprised 65% of the strides and hopping 35%. The amount of hopping increased gradually until it was continuous at 6 km h<sup>-1</sup>. (Preliminary tests with other *N. cervinus* suggest that this is the common pattern.) For the third animal trotting did not change to galloping until ~ 2.5 km h<sup>-1</sup>, and significant hopping was not evident until 6 km h<sup>-1</sup>. Even at 7 km h<sup>-1</sup> hops only comprised 45% of strides. Why this animal should be different from the others was not obvious; initially it was rather recalcitrant, but with training it performed well. Hopping (or saltatorial bipedal locomotion) seems to be associated with a marked shift in the energy costs of locomotion from the patterns established for running quadrupeds<sup>2</sup> and bipeds<sup>3</sup>. This results in a much lower overall cost of locomotion in the small hopping mice and lower cost at moderate speeds in the large kangaroos.

Our data demonstrate some of the advantages of hopping, particularly for a small rodent living in a desert where food is sparse and cover for protection from predators is limited. Although reasons may also be suggested for the selective advantage of hopping for kangaroos<sup>1</sup>, there is still the question why kangaroos are the only large animals with saltatory locomotion and our experiments give some insight here also. At low speeds the *N. cervinus* largely follow the energy predictions of ref. 2 and when they diverge from this pattern they immediately achieve lower locomotory costs. Kangaroos, however, have much higher costs than predicted for quadrupeds at low speeds, and it is not until moderately high speeds (17–18 km h<sup>-1</sup> for an 18-kg animal) that the costs of hopping become less than those predicted.

In an analysis of the mechanical characteristics of very long leaps of kangaroos, Badoux<sup>4</sup> observed that, to develop the necessary acceleration, the feet must exert a force directly proportional to the animal's mass and inversely proportional to the length of leg. Taylor *et al.*<sup>5</sup> reiterated that the work involved in lifting 1 g of tissue a given vertical height is the same for small animals as for large, if the mechanical efficiency of the muscle of both animals is the same. Because both the resting metabolic rate and the relative cost of locomotion decrease with increasing weight, larger animals have to increase their metabolism by a proportionately greater amount than do small animals to lift a given weight of tissue a given height. The energetic implications of this for hopping bipeds are obvious, particularly at low speeds when elastic storage of energy would be low. The pentapedal gait of large kangaroos at slow speeds may reflect a partial answer to this problem, since anatomical specialisations limit the use of quadrupedal locomotion.

Howell<sup>6</sup> has suggested that biped hopping is an extreme form of the gallop. This is possibly illustrated by the data on the mouse with the higher oxygen consumption. While this animal did not commence significant hopping until ~ 6 km h<sup>-1</sup>, stride frequency and oxygen consumption reached

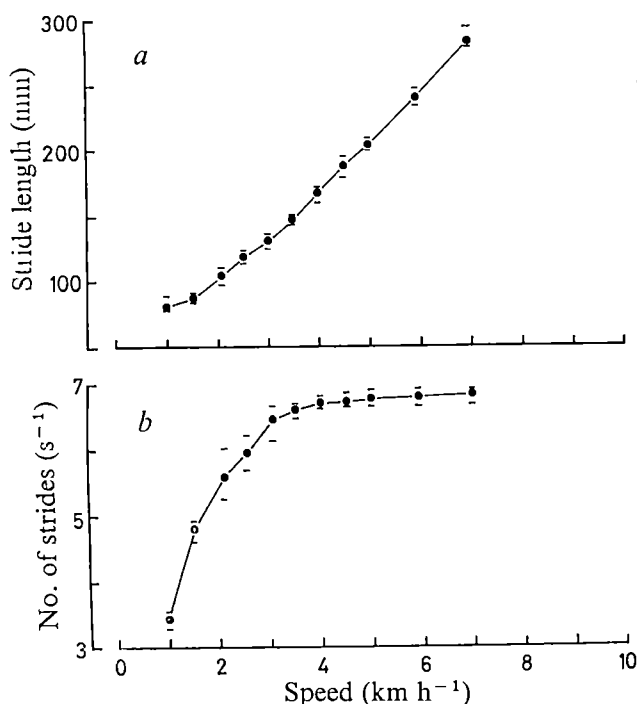


Fig. 2 a, Stride length; b, stride frequency as a function of speed for *N. cervinus* during steady-state locomotion. The values shown in both graphs are the mean values for the three individuals and the horizontal bars indicate the range of values.

their plateaux at the same speed as for the other hopping mice, 2.5–3.0 km h<sup>-1</sup>. Stride frequency tends to stabilise in most animals once they commence galloping<sup>7</sup>. Consequently it may be that a similar but smaller change in oxygen consumption occurs in other species at speeds above the transition from trotting to galloping, but that this is emphasised in species with anatomical specifications such as those for hopping. This needs further investigation since the data with which Taylor *et al.*<sup>2</sup> established a relationship between the energetic costs of locomotion and body weight were mostly collected at speeds below the trot–gallop transition. This was particularly the case for their kangaroo rats (*Dipodomys merriami* and *D. spectabilis*).

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## Mutants of *Klebsiella pneumoniae* resistant to several antibiotics

SPONTANEOUS antibiotic-resistant mutants that arise in bacterial populations are usually only resistant to the particular antibiotic used in the culture medium used for their isolation. In the experiments described here, however, mutants of *Klebsiella pneumoniae* resistant to chloramphenicol, sodium nalidixate and trimethoprim, three unrelated antibiotics, were obtained by including any one of the antibiotics in the culture medium. Because these multiply resistant mutants were commonly found in the alimentary tract of animals experimentally infected with antibiotic-sensitive *K. pneumoniae* and then fed on antibiotic-containing diets they may be clinically important.

Mutants resistant to antibiotics other than trimethoprim were obtained by spreading 0.75 ml of a 24-h broth culture containing ~ 5 × 10<sup>8</sup> viable organisms per ml over the surface of a well-dried plate of MacConkey's agar containing antibiotic (20 µg ml<sup>-1</sup>). The plates were incubated at 37 °C for 24 h and colonies that grew on them purified by plating on ordinary MacConkey's agar. Washed broth cultures and Sensitest agar (Oxoid, CM409) containing trimethoprim (3 µg ml<sup>-1</sup>) were used for obtaining mutants resistant to this antibiotic. Antibiotic sensitivity tests were performed by placing disks containing streptomycin (25 µg), tetracycline (30 µg), chloramphenicol (30 µg), ampicillin (25 µg), neomycin (30 µg), spectinomycin (30 µg), rifampicin (50 µg), sodium nalidixate (30 µg), sulphafurazole (50 µg), trimethoprim (1.25 µg) and furazolidone (15 µg) on the surface of plates of Sensitest agar spread with well diluted broth cultures; the plates were incubated at 37 °C for 24 h and read.

When mutants resistant to sodium nalidixate (*nal*<sup>r</sup>), chloramphenicol (*cmp*<sup>r</sup>) and trimethoprim (*tmp*<sup>r</sup>) were isolated from 27 of 32 epidemiologically unrelated strains of *K. pneumoniae* belonging to 20 different capsular types, they were found to be resistant not only to whichever of these three antibiotics were incorporated in the selection

medium but also to the other two; 50–200 colonies grew on each of the plates used. All the colonies on the chloramphenicol and trimethoprim plates that had been inoculated with five of the strains were found by replica plating to be *cmp*<sup>r</sup> *nal*<sup>r</sup> and *tmp*<sup>r</sup>. So were all those on the sodium nalidixate plates inoculated with three of these strains; a few of the 50 or so colonies that grew on each of the sodium nalidixate plates inoculated with the other two strains were *nal*<sup>r</sup> only, the remainder being resistant to the three antibiotics. Disk tests indicated that the sensitivity of these *cmp*<sup>r</sup> *nal*<sup>r</sup> *tmp*<sup>r</sup> mutants to tetracycline, streptomycin, neomycin, spectinomycin, rifampicin, polymyxin, sulphafurazole and furazolidone had not altered appreciably; the strains from which they were derived were resistant to ampicillin. Using very light inocula, six of the multiply resistant mutants were passaged ten times in 10-ml amounts of broth at 37 °C, each passage occupying 24 h; their antibiotic-resistance pattern did not alter as a result. Attempts to transfer the resistance of four of them to *Escherichia coli*, K12 were unsuccessful. The colonial morphology of the mutants was the same as that of their parent strains, and so was the capsular type of all five examined by Dr Ida Ørskov and the virulence for mice by intraperitoneal injection of the only one tested. Like their parent strains, the mutants were prototrophic.

**Table 1** Minimum inhibitory concentration of antibiotics for *K. pneumoniae* mutants and their parent strains

Antibiotic	Median MIC (µg ml <sup>-1</sup> ) for	
	parent strains	mutants
Sodium nalidixate	3	100
Chloramphenicol	3	100
Trimethoprim	0.4	25
Tetracycline	3	12
Sulphafurazole	50	100
Furazolidone	0.8	1.5
Streptomycin	1.5	1.5
Neomycin	1.5	1.5
Spectinomycin	12	12
Rifampicin	25	25
Polymyxin	0.8	0.8

Ten mutant and ten parent strains were examined. The minimum inhibitory concentration (MIC) was determined by inoculating small areas of plates containing twofold falling concentrations of antibiotic in Sensitest agar with approximately 200 viable organisms and then incubating the plates at 37 °C for 24 h. The lowest concentration of antibiotic that prevented growth was recorded as the MIC.

The minimum inhibitory concentrations of sodium nalidixate, chloramphenicol and trimethoprim for the mutants were 25–50 higher than those for their parent strains (Table 1). Those of tetracycline, sulphafurazole and furazolidone, but not of streptomycin, neomycin, spectinomycin, rifampicin and polymyxin, were slightly higher for the mutants than those for their parent strains.

Mutants resistant to streptomycin, spectinomycin and rifampicin were isolated from three *K. pneumoniae* strains; they were resistant only to the antibiotic used in their isolation.

To determine whether a multiply resistant *K. pneumoniae* flora could emerge *in vivo* during the administration of only one antibiotic, groups of 15 1-d-old chicks were given orally 10<sup>8</sup> viable organisms of a spectinomycin-resistant mutant of a *K. pneumoniae* strain and then fed freely on diets containing sodium nalidixate, chloramphenicol or trimethoprim (100 or 500 mg kg<sup>-1</sup>) or no antibiotics for 10 d. Cloacal swabs taken at intervals from the chicks were each smeared over the surface of a MacConkey plate and a Sensitest plate, both containing spectinomycin (20 µg ml<sup>-1</sup>), and a sodium nalidixate disk and a chloramphenicol disk were placed on the MacConkey plate and a trimethoprim disk was placed on the Sensitest plate. The plates



**Table 2** Faecal excretion of antibiotic-resistant organisms by chicks fed diets containing chloramphenicol, trimethoprim or sodium nalidixate after receiving orally a culture of *K. pneumoniae* sensitive to these antibiotics

Antibiotic administered	Time after commencement of administration	No. out of 15 chicks excreting <i>K. pneumoniae</i> organisms that were		
		only the antibiotic administered	resistant to chloramphenicol trimethoprim and sodium nalidixate	all sensitive
Chloramphenicol	4	0(0)	4(13)	11(2)
	8	0(0)	9(10)	4(1)
	17*	0(0)	1(5)	7(3)
Trimethoprim	4	3(8)	7(4)	6(5)
	8	12(11)	7(6)	1(0)
	17*	14(9)	1(1)	1(0)
Sodium nalidixate	4	0(0)	0(0)	15(15)
	8	0(0)	0(0)	13(8)
	4	0(0)	0(0)	15(15)
None	8	0(0)	0(0)	15(15)

Chicks received antibiotic in a concentration of 100 mg kg<sup>-1</sup> and (figures in parentheses) 500 mg kg<sup>-1</sup>.

\*Seven days after the feeding of antibiotic-containing diets ceased.

were incubated at 37 °C for 24 h and those with colonies of *K. pneumoniae* organisms on them that were resistant to sodium nalidixate, chloramphenicol and trimethoprim were recorded. These organisms were found in the faeces of many of the chicks fed diets containing chloramphenicol or trimethoprim (Table 2); from some of them they were the only kind isolated. Organisms of the infecting strain resistant to trimethoprim only were also common in the faeces of most of the chicks in the trimethoprim group. The multiply resistant organisms were also isolated from a few of the chicks in the chloramphenicol and trimethoprim groups 7 d after withdrawing the antibiotic-containing diets; at this time most of the chicks in all the groups had no or very few organisms of the infecting strain in their faeces. No resistant organisms of this strain were found in the faeces of any of the chicks fed diets containing sodium nalidixate or no antibiotics. Surprisingly, on account of the ease with which they can be isolated *in vitro*, no *nal*<sup>r</sup> *E. coli* were found in the faeces of chicks in the sodium nalidixate group.

Antibiotic-resistant mutants of enterobacteria other than *K. pneumoniae* that were available in this laboratory were examined for resistance to antibiotics different from the ones used in their isolation. In addition, those whose parent strains were prototrophic were cultured on a medium consisting of (g l<sup>-1</sup>) K<sub>2</sub>HPO<sub>4</sub> 7; KH<sub>2</sub>PO<sub>4</sub> 3; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1; NaCl, 5; glucose, 5; agar, 15 to determine whether they too were prototrophic. The antibiotic that had been used to isolate the mutants from different pathogenic and non-pathogenic strains of *E. coli* of human, bovine, porcine and fowl origin was sodium nalidixate (106), streptomycin (40), ampicillin (14), spectinomycin (10) and rifampicin (1), from different *Salmonella* strains was sodium nalidixate (24), streptomycin (18), ampicillin (1), spectinomycin (3) and rifampicin (5) and from different *Shigella flexneri* and *S. sonnei* strains was sodium nalidixate (8), streptomycin (5), spectinomycin (1) and rifampicin (2). All these 238 mutants were only resistant to the antibiotic used in their isolation and all the 214 with prototrophic parents were also prototrophic.

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## Selective entry of immunoblasts into gut from intestinal lymph

THE observation<sup>1</sup> that the lymphoid blast cells in the thoracic duct lymph of rats homed preferentially to the lamina propria of the small gut after being injected intravenously has received ample confirmation<sup>2-4</sup>. But although the thoracic duct lymph of rodents comes principally from the intestine there must also be a significant contribution from other viscera as well as from the caudal somatic lymph nodes. It follows that the large lymphoid immunoblasts present in thoracic duct lymph may have come from several sources, and it has been shown that their numbers increase after antigens have been injected subcutaneously into the hindquarters<sup>5</sup>. Are the immunoblasts that home to the small gut selected randomly, or do they have some special feature which mediates their extravasation in the gut? Straightforward immunological factors such as the specificity of the immunoblasts or the presence of antigens in the gut do not seem to be of primary importance<sup>6,7</sup>.

Immunoblasts teased from somatic lymph nodes cannot home to the gut nearly as well as those from mesenteric nodes or thoracic duct lymph<sup>2,8</sup> but the performance of immunoblasts which normally enter the lymph from somatic nodes has not been measured. By cannulating appropriate lymph ducts in experimental sheep we have studied the problem directly and our results are reported here.

Yearling sheep were anaesthetised and polyvinyl cannulae inserted into the intestinal lymph duct and an efferent duct of one of the peripheral, somatic lymph nodes (popliteal, prefemoral or prescapular). After the operation the conscious sheep were placed in metabolism cages and the lymph was collected quantitatively into sterile, heparinised, plastic bottles<sup>9-11</sup>. In these relatively young sheep up to 15% of the white cells in the intestinal lymph were immunoblasts, but in the efferent lymph from somatic nodes, immunoblasts accounted for 2% or less of the white cells. For this reason 2 ml of a mixture of suspensions of killed bacteria (*Corynebacterium parvum*, *Brucella abortus* and *Salmonella typhi* O and H, Wellcome Research Laboratories) was injected subcutaneously into the drainage area of the cannulated node so that 4 d later, when the immune response was greatest, 20-40% of the cells in the efferent lymph consisted of immunoblasts<sup>12,13</sup>. An 8-h collection of either intestinal or efferent lymph was then incubated for 1 h at 38 °C with <sup>125</sup>I-iododeoxyuridine (IUDR, Radiochemical Centre, Amersham) at a final concentration

**Table 1** Percentages of injected radioactivity present in tissues of sheep 20 h after injection of autochthonous immunoblasts collected from intestinal lymph or efferent lymph of somatic lymph nodes and labelled *in vitro* with  $^{125}\text{I}$ UdR

Type of labelled cells injected	Immunoblasts from efferent lymph of somatic nodes				Immunoblasts from intestinal lymph				
	1	3	5	7	2	4	6	8	10
Experiment no.									
Small intestine	0.9	0.4	0.8	0.6	35.3	30.5	27.8	27.0	28.4
Spleen	16.0	16.8	21.0	19.0	0.2	1.4	0.3	1.4	0.4
Mesenteric lymph nodes	0.1	0.1	0.2	0.1	1.8	3.4	2.7	2.9	3.4
Somatic lymph nodes	1.3	2.6	0.9	0.8	0.2	1.1	0.3	0.7	0.6

of  $0.1 \mu\text{Ci ml}^{-1}$ . The total number of white cells in the collections varied between  $10^6$  and  $10^{10}$  and included 10–40% immunoblasts; autoradiographs showed later that 70–80% of the latter had labelled nuclei. The labelled cells were then washed once and returned to the sheep by intravenous injection. The sheep was killed 20 h later and selected organs were removed, cut into small pieces and loaded into counting vials so that the gamma emission of each organ could be measured in a scintillation spectrometer. The relevant results from nine such experiments are shown in Table 1.

Clearly immunoblasts generated in somatic nodes did not enter the small gut but tended to localise in the spleen and, incidentally, did not reappear in significant numbers in samples of either lymph or blood. Conversely, immunoblasts generated in the lymphoid tissue associated with the gut avoided the spleen and localised mainly in the small gut; up to a further 10% of the injected cells were recovered in the intestinal lymph during the course of the experiments. The latter observation shows that, as in studies on rats<sup>3,4,6</sup> most radioactivity remained associated with intact immunoblasts throughout the experiment. Unfortunately, it is not practical to prepare representative autoradiographs from the major organs of an adult sheep, and so little can be said about the microanatomical localisation of the labelled cells—we assume that it is generally similar to that found in the rat<sup>3,4</sup>.

It was suggested, on *a priori* grounds, that immunoblasts which enter the gut must be IgA secretors<sup>4</sup> and there is evidence for this view<sup>8,14</sup>. In the experiments reported here we found, by immunodiffusion tests, that detergent extracts of washed cells from intestinal lymph always contained IgA as the major immunoglobulin. Extracts of lymph cells from stimulated somatic nodes contained mainly IgG, with some IgM, but we found no IgA. Furthermore, the immunoblasts from somatic nodes did not acquire the ability to home to gut even when they had been collected and incubated in isologous intestinal lymph plasma, which is rich in IgA<sup>15</sup>.

These findings support the view that one of the intrinsic cellular factors which mediate the extravasation of immunoblasts in the small gut is the production of IgA or a molecule associated closely with it. Certainly the view<sup>3</sup> that lymph-borne immunoblasts from any source may extravasate into the gut is wrong in any general sense.

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## Protection of mice against *Babesia* and *Plasmodium* with BCG

*Babesia* spp. are haemoprotozoans, and some cause diseases of considerable economic importance in cattle. Complete protection against challenge has been recorded only in animals recovered from artificial<sup>1</sup> or natural infections with a virulent strain of the organism. We have achieved the same result with *B. microti* and *B. rodhaini*, two of the species which parasitise mice, by previous infection with *Mycobacterium bovis* strain BCG (Bacillus Calmette-Guérin). Evidence is provided that neither antibody specific for surface antigens of the parasites nor increased phagocytic capacity is responsible for this protection. We suggest that BCG protects by increasing the release of non-antibody soluble mediators of immunity postulated to cause the death of *B. microti* inside red cells<sup>2</sup>. Preliminary observations on protection against *Plasmodium berghei yoelii* and *P. vinckei* are also presented.

Eight female CBA mice 6 weeks old were given  $2 \times 10^7$  live BCG (Glaxo strain) intravenously. Two weeks later  $10^6$  *B. microti* were injected intraperitoneally into these mice and into an equal number of controls. In the mice that received BCG a few parasites were seen in tail blood smears after about a week, but none thereafter during an observation period of more than 100 d. The control group showed the usual primary infection curve for this parasite (Fig. 1a).

Subsequently a group of nine 6-week-old female CBA mice were given  $10^6$  *B. microti* intraperitoneally 28 d after they had received  $2 \times 10^7$  live BCG intravenously. For 155 d no parasites were observed in smears from these mice, even though further challenges of  $2 \times 10^6$ ,  $3 \times 10^6$ ,  $2.5 \times 10^7$  and  $10^6$  organisms were given 14, 25, 50 and 100 d, respectively, after the initial infection. Indeed, absence of recrudescence after splenectomy of three mice on day 40, and failure to transfer infection with the spleens from these animals, suggest that a sterile immunity was achieved.

All of 57 mice, including males, have now been completely protected in eight experiments. Eight of these mice withstood  $7.5 \times 10^8$  organisms. This is a massive dose compared with challenges usually given in protection experiments with blood parasites<sup>3–6</sup> and indicates the strength of

the protection conferred by BCG. The dose used produced a 2% parasitaemia within hours of being given intraperitoneally, but the count did not rise further. As healthy parasites became fewer over the next 2 d the inclusion bodies shown by electron microscopy to be degenerate intraerythrocytic parasites<sup>2</sup> appeared. These were in sufficient numbers to account for at least most of the

original healthy parasites. Thus *B. microti* die inside the red cells of mice protected with BCG.

Indirect fluorescent antibody titres to *B. microti* antigen were negligible in sera from mice given BCG 26 d previously and not challenged with *B. microti*, and also in serum from mice protected with BCG (Table 1). This test detects all antibody specific for surface antigens of the parasite. Furthermore, giving 3 mg silica intravenously (Doröntrop Quartz No. 12,  $<5\ \mu\text{m}$ ) either before or after challenging two groups of six BCG-immunised mice with *B. microti*, which would be expected to decrease antibody production and phagocytic activity, respectively<sup>7</sup>, did not decrease the protection that BCG gave. Thus this protection is unlikely to be due to cross-reacting antibodies, or enhancement of either antibody production or phagocytic activity. In any event the intraerythrocytic degeneration of the parasites precludes their death inside macrophages.

Ten female CBA mice in two experiments were completely protected against *B. rodhaini* (Antwerp Strain), which is invariably fatal in unprotected mice, by giving them live BCG intravenously 3 weeks before challenge (Fig. 1c). When protection of one of these mice lapsed temporarily from day 20 we obtained light microscopical evidence of intraerythrocytic death of *B. rodhaini* also. Again we used  $2 \times 10^7$  BCG and  $10^6$  parasites. This number of organisms killed the four control mice on day 9.

Similarly, in one preliminary experiment we were able to protect four out of four female CBA mice against  $10^6$  *P. berghei yoelii* (Strain 17X) by intravenous injection of  $2 \times 10^7$  live BCG 1 month beforehand (Fig. 1d). Some male CBA mice (two out of four) were less well protected. An appreciable degree of protection, again with light microscopical evidence of intraerythrocytic death of parasites, has also been achieved in eight mice with *P. vinckei* in the same conditions (Fig. 1e). This parasite is normally lethal in mice, and killed the four controls on day 8, but the protected group survived.

Vaccines incorporating killed *Mycobacterium* sp. and parasites, given intradermally, subcutaneously or intramuscularly, have been used against *B. argentine*<sup>3</sup> and *P. knowlesi*<sup>6,8,9</sup>. All except the one containing merozoites<sup>6</sup> were only moderately successful. Killed *Mycobacterium* sp. alone has not been protective, perhaps because it was never given intravenously. Live BCG, on the other hand, has been reported to protect against some protozoa, the success varying with the route of administration. Thus intraperitoneal live BCG has been unable to protect mice against *Trypanosoma cruzi*<sup>10</sup> or *Toxoplasma gondii*<sup>11</sup>. Likewise, live BCG injected into the retrobulbar space of rabbits did not protect the eye from *Toxoplasma gondii*<sup>12</sup>. In contrast, pretreatment with intravenous live BCG reduced the number of circulating *Trypanosoma cruzi* in mice<sup>13</sup> and protected rabbits' eyes from *Toxoplasma gondii*<sup>12</sup>. In neither case, however, was the protection as strong as that which we have observed against *B. microti*, *B. rodhaini*, *P. berghei yoelii* or *P. vinckei*. This may be simply a question of timing and dose of BCG rather than any fundamental difference between the protection afforded against the different protozoa.

BCG provides nonspecific resistance in a wide variety of diseases, both neoplastic<sup>14,15</sup> and infectious<sup>16,17</sup>. In certain

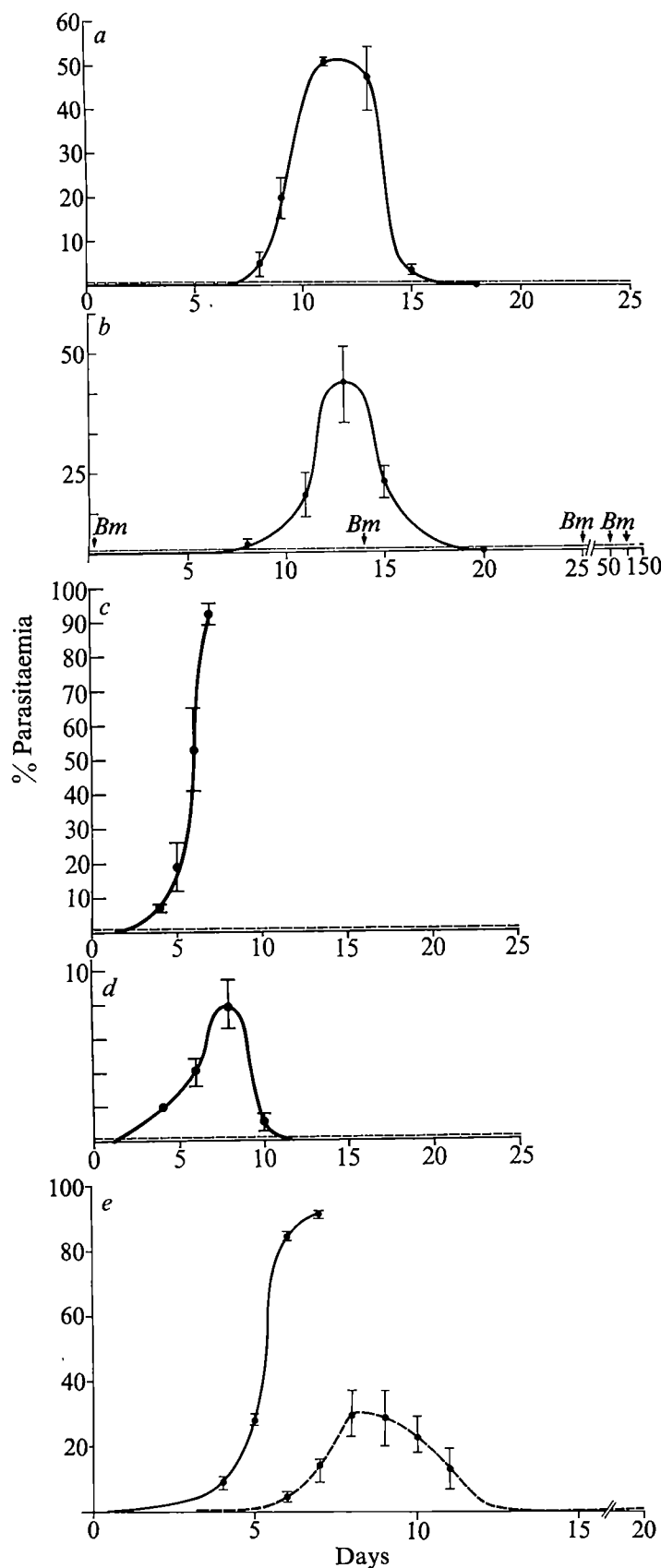


Fig. 1 a, Course of infection of mice given  $2 \times 10^7$  live BCG intravenously, and  $10^6$  *B. microti* 14 d later. — — —, BCG-vaccinated; —, non-vaccinated. b,  $10^6$  *B. microti* given 28 d after  $2 \times 10^7$  live BCG intravenously. Bm, unsuccessful rechallenges; — — —, BCG-vaccinated; —, non-vaccinated. c,  $10^6$  *B. rodhaini* given 21 d after  $2 \times 10^7$  live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. d,  $10^6$  *P. berghei yoelii* given 28 d after  $2 \times 10^7$  live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. e,  $10^6$  *P. vinckei* given 28 d after  $2 \times 10^7$  live BCG intravenously. — — —, BCG-vaccinated; —, non-vaccinated. s.d. as shown.

conditions BCG can increase antibody production<sup>18</sup> and make macrophages hyperactive<sup>19</sup>. From our evidence it seems unlikely that either of these two mechanisms, or cross-reacting antibodies, can explain the protection observed. The occasional appearance of parasites in smears from all the mice infected intraperitoneally with *B. microti* 14 d after BCG inoculation and in some of those protected against *B. rodhaini* eliminates the possibility that activated peritoneal macrophages<sup>20</sup> prevented the parasites reaching the circulation.

Immunity to *Babesia* is not yet understood. Thymus-derived lymphocytes are necessary for recovery from infection<sup>21,22</sup>, but their role is uncertain. Transfer of large volumes of recovery serum have yielded inconsistent results<sup>4,23-25</sup>; thus it seems unlikely that T lymphocytes merely help in the production of antibody that is free in the serum. Furthermore there is no evidence that T lymphocytes can kill *Babesia* parasites or infected erythrocytes by contact lysis.

**Table 1** Anti-*Babesia* antibody levels

Time serum taken	Titre
26 d after BCG alone	0
12 and 20 d after <i>B. microti</i> (BCG 14 d before <i>B. microti</i> )	1:20
14 d after <i>B. microti</i> (BCG 28 d before <i>B. microti</i> )	1:20
Recovered controls	1:2,560

Mice given BCG, BCG and *B. microti*, and *B. microti* alone. Antibody levels detected by an indirect fluorescent antibody test. Serum from at least six mice pooled for each reading.

Another hypothesis is that T lymphocytes are necessary for the release of a non-antibody mediator of immunity. Indeed, the massive T-cell activation in spleens of mice infected with *P. berghei* yoelii<sup>26</sup> could be explained on this basis, as the authors remarked. There are many such products of activated lymphoid cells which exhibit various activities *in vitro*; some, for instance, cause intracellular death of *Toxoplasma gondii*<sup>27</sup>, and others of *M. tuberculosis*<sup>28</sup>.

Among these mediators are the substances with interferon and migration-inhibition activities present in the serum of BCG-infected mice after stimulation either specifically or with certain unrelated antigens<sup>29</sup>. As well as protecting mice from *M. tuberculosis*<sup>30</sup>, these substances can inhibit various unrelated organisms *in vitro*<sup>31</sup>. The finding that BCG must be given intravenously for appreciable release of the mediators of this inhibition<sup>30</sup> is consistent with the requirement for this route of administration to protect against certain protozoa<sup>10-13</sup>, and our failure to protect with subcutaneous BCG. Our results are also in keeping with the original proposal by Mackaness<sup>32</sup> that cellular immunity, although specifically induced, may be non-specifically expressed; he also suggested that this could be accomplished by migration inhibition factor<sup>33</sup>. Furthermore, interferon activity has been reported in the serum of mice infected with *P. berghei*<sup>34</sup> and interferon inducers protect mice against this organism<sup>35,36</sup>.

For these reasons, and in view of the intraerythrocytic death of parasites both in normally recovering<sup>2</sup> and BCG-protected mice, we consider that the most likely mechanism of immunity to these two genera of parasites in mice is the release of such a mediator, the output of mediator(s) being increased, and thus protection augmented, by previous infection with BCG.

The duration and details of the nature of this protection remain to be established. Nevertheless, in addition to giving a greater understanding of the basic mechanism of immunity involved, this approach may lead to a practical method of stimulating immunity against these two genera

of blood parasites. Further, its nonspecificity promises activity against a range of species and strains.

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## Contact-induced spreading is a new phenomenon depending on cell-cell contact

THERE is firm evidence that the characteristic changes of cell behaviour *in vitro* described as contact inhibition of locomotion<sup>1</sup>, contact inhibition of extension<sup>2</sup> and contact inhibition of phagocytosis<sup>3</sup> occur as a direct result of cell-cell contact. In addition it has been suggested<sup>4</sup> that the inhibition of both pinocytosis<sup>5</sup> and blebbing<sup>6</sup>, in those regions of the margins of cells in contact with other cells, may also be a direct result of cell-cell contact. These examples may well be part of a general class of cell-contact-dependent phenomena which could play an important role in cellular interactions. I report here another contact-dependent reaction which takes place between chick embryo pigmented retina epithelial (PRE) cells in culture. In brief, it can be shown that the degree of polarisation exhibited by these cells and the degree to which they are spread on their substrate is influenced profoundly by whether or not they are in contact with other cells.

Observations<sup>7</sup> of cultured PRE cells moving on a plane substrate have shown that collisions between the cells usually result in the development of stable contacts between the cells, so that islands and sheets of cells are eventually formed from a culture which consisted initially of isolated



single cells. In addition it has been found that, whereas isolated PRE cells lacking contacts with other cells are usually only poorly spread on the substrate and only infrequently display fibroblast-like leading lamellae, those PRE cells incorporated into islands of cells are much more extensively spread and display typical leading lamellae if marginally positioned in an island<sup>7</sup>. My preliminary observations suggested that as a previously isolated and poorly spread cell made contact with another cell or island of cells, it underwent a striking change in morphology and behaviour to resemble the cells of the island it had joined<sup>7</sup>. The investigation reported here confirms this observation and demonstrates that this change in morphology is dependent on cell-cell contact.

A single-cell suspension of 10-d chick embryo PRE cells was prepared and plated out on to collagen-coated coverslips as previously described<sup>8</sup>. The cultures were incubated at 37 °C for 5 h to allow the cells to attach to the substrate before being prepared for photography. A field of cells was selected and photographed at 30-min intervals for periods of up to 6 h. A selection from a representative series of such photographs is shown in Fig. 1.

The characteristic difference in morphology between isolated cells and cells in contact with other cells is clearly seen in Fig. 1a. The isolated cell illustrated is clearly only poorly spread on the substrate and as a result its nucleus is obscured by pigment granules. The general appearance of such isolated cells suggests that they are 'unpolarised' in that they usually lack a fibroblast-like leading lamella and they display vigorous and apparently uncoordinated blebbing activity at their margins. Although the cells are poorly spread they clearly adhere relatively well to the substrate since cell detachment was never observed although all observations were made on inverted cultures. In addition I have found that such cells can withstand vigorous washing

and perfusion without detaching from the substrate (unpublished observations). By contrast to the isolated cells, those incorporated into islands are much more extensively spread on the substrate (Fig. 1a). Cells that are marginally positioned in an island exhibit clear 'polarisation' in that they display typical fibroblast-like leading lamellae from those parts of their margin not in contact with other cells. Blebbing by these well spread cells is only very rarely observed.

The sequence of photographs shown in Fig. 1 demonstrates the striking change in morphology shown by an isolated cell after colliding with an island of cells. The first signs of this change are evident within 30 min of the collision (Fig. 1b). By this time the cell has begun to spread more extensively on the substrate and as a result its nucleus has become less obscured by pigment granules, blebbing has decreased and the first signs of polarisation are evident. Within 90 min of the collision the cell closely resembles the other cells making up the island, blebbing has ceased, the cell is well spread on the substrate and a leading lamella is beginning to develop from the area of its margin not in contact with other cells (Fig. 1c). With further time the cell becomes indistinguishable from its neighbours in the island (Fig. 1d).

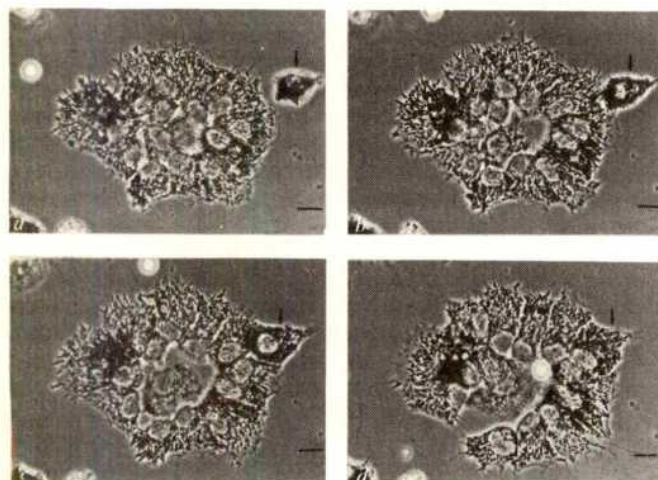
**Table 1** Comparison of the area of substrate occupied by single cells and cells in two-cell islands

	Mean cell spread area $\pm$ s.e. ( $\mu\text{m}^2$ )	n	
Single cells	315.4 $\pm$ 27.5	25	
*Two-cell islands	496.2 $\pm$ 38.8	25	$P < 0.001$

\*The area of complete two-cell islands was measured and the mean area occupied by the individual cells was obtained by division.

Five cultures of PRE cells were prepared essentially as described in the legend to Fig. 1, but the original cell suspension was adjusted to  $3.2 \times 10^5 \text{ ml}^{-1}$  before being plated out. Medium, substrate, culture vessels, sample size and incubating conditions were as described in the legend to Fig. 1. The cultures were incubated for 24 h and then the coverslips with attached cells were removed, fixed with formol saline and stained with Harris' haematoxylin. The areas of five single cells and five two-cell islands in each of the fixed cultures was measured using the Curtis<sup>10</sup> modification of the Chalkley<sup>11</sup> random array method. Sufficient estimates of each measured area were obtained to ensure that the final estimate had a standard error of 5% or less.

There seems little reason to doubt that this change in the behaviour and morphology of a previously isolated cell is a direct response to contact with other cells. Alternative explanations in terms of adaptation to culture conditions or recovery from cell dissociation procedures are unlikely since the characteristic differences between isolated cells and those in contact with other cells are maintained for several days in culture. Equally it seems unlikely that the observations can be explained in terms of local diffusion gradients surrounding the islands of cells. Isolated PRE cells move only rather slowly and inefficiently<sup>7</sup>; thus an isolated poorly spread cell may lie within 5–10  $\mu\text{m}$  of the edge of an island of well spread cells yet it will display no signs of spreading or polarisation unless it establishes visible (to the light microscope) contact with the island. This observation could be reconciled with an explanation in terms of diffusion gradients only if such gradients were extremely local in effect. Very short range gradients caused by diffusion boundary layer effects have been proposed to influence the division of cells in culture<sup>9</sup>. Such a system seems unlikely to be operating here, however, since it can be shown that the individual cells comprising a two cell island occupy a significantly greater area of the substrate than do single isolated cells (Table 1). In fact the data in Table 1 indicate that a collision between two previously isolated cells results in both cells increasing by approximately 50% the area of the substrate that they occupy individually. It is difficult to



**Fig. 1** Contact-induced spreading of a PRE cell. A single cell suspension was prepared from the pigmented retina of 10-d chick embryos as previously described<sup>8</sup>. The cells were suspended in Ham's F10 supplemented with 10% chick serum at pH 7.6 and the concentration was adjusted to  $4.8 \times 10^5 \text{ ml}^{-1}$ . Samples of 0.2 ml of the suspension were plated out into 9-mm diameter glass rings 1 cm high, attached with sterile silicone grease to collagen-coated coverslips<sup>8</sup>. Cultures were incubated at 37 °C under a gas phase of 5%  $\text{CO}_2$ , 20%  $\text{O}_2$  and 75%  $\text{N}_2$ . After 5 h the glass rings were removed and the coverslips with attached cells were converted to inverted filming slides for photography. Photographs were obtained using a Zeiss photomicroscope fitted with phase contrast optics; the cultures being maintained at 37 °C by an air curtain incubator. a–d, Series of photographs of the same microscope field to illustrate the spreading of a single PRE cell (arrow) after contact with an island of cells. a, After 5 h in culture, arbitrarily defined as 0 time; b, 0 + 30 min; c, 0 + 90 min; d, 0 + 240 min. See text for details. The scale bar represents 10  $\mu\text{m}$ .



see how this response at the single cell contact level could be explained in terms of any diffusion gradient theory.

It seems clear that the marked change in morphology and behaviour shown by isolated PRE cells in response to a collision with an island of cells is dependent on cell contact. Since the most striking aspect of the response is the extensive spreading of the cell I propose that the phenomenon be called contact-induced spreading.

There have been no reports of a reaction similar to contact-induced spreading occurring in fibroblastic cell types but there is at least preliminary evidence to suggest that the phenomenon may not be restricted to PRE cells. Trinkaus<sup>12</sup> noted that while isolated *Fundulus* gastrula cells in culture were normally only poorly spread on the substrate, those incorporated into islands of cells were spread extensively. Additionally, a reaction similar to contact-induced spreading has been observed in cultured chick liver parenchyma cells<sup>13</sup> and cultured limpet haematocytes (T. A. Partridge, personal communication). It is interesting that all these examples are of cells that assume an epithelioid morphology in tissue culture and it seems possible therefore that contact-induced spreading may be a characteristic of cultured epithelial cells.

The mechanism of contact-induced spreading is obscure but it seems clear that it must involve changes in the adhesion of the cell to the substrate since the area of cell-substrate apposition is considerably increased as a result of the reaction. It seems therefore that the development of a cell-cell contact can affect cell-substrate adhesion. A similar conclusion was reached by Trinkaus from his observations of cultured *Fundulus* gastrula cells<sup>12</sup>.

It is likely that contact-induced spreading also involves a change in the locomotory behaviour of the cell since as a result of the reaction a previously unpolarised blebbing cell develops a fibroblast-like leading lamella and, from time-lapse film observations<sup>7</sup>, appears to be attempting to move away from the islands it has joined. It is apparent from Fig 1 that the phenomenon also involves the development of extensive regions of contact, and presumably therefore adhesion, between the previously isolated cell and its neighbours in the island. Although it has been shown that ultrastructural contact specialisations develop between PRE cells within 24 h in culture<sup>7</sup>, it is not known whether or not these develop rapidly enough to play any role in contact-induced spreading.

Contact-induced spreading seems to be another example of the group of cellular interactions dependent on cell-cell contact. Whether or not the various members of this group share common mechanisms remains to be established. The findings reported here, however, again emphasise the importance of cell-cell contacts in regulating the behaviour of cells, at least *in vitro*.

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## Chronic suppression of immunoglobulin allotype production in adult congenic mice

MOUSE allotypes refer to the inheritance of antigenic differences on the constant region of immunoglobulin (Ig) heavy chains ( $C_H$ ). As such, allotypes are used as genetic markers to distinguish allelic  $C_H$  genes, the expression of which is codominant in heterozygous mice<sup>1,2</sup>. The expression of a paternal  $C_H$  gene, however, can be suppressed in heterozygote offspring if the mother has previously been made immune to the allotype encoded by the paternal gene<sup>3</sup>. Neonatally induced suppression of this sort is known to lead to (or result in) the generation of allotype-specific suppressor T lymphocytes which directly or indirectly prevent allotype production by B lymphocytes<sup>4-6</sup>. In contrast to this we have shown that allotype suppression can also be initiated in adult mice, that is in BALB/c mice that are congenic for the C57BL allotype (C.B-17 mice)<sup>1</sup>. When X-irradiated C.B-17 mice (550 r.) are injected with thymocytes from BALB/c donors immune to the C57BL allotype, IgG<sub>2a</sub> allotype production in the C.B-17 recipients becomes chronically suppressed<sup>7</sup>. We now report that allotype suppression in C.B-17 mice is mediated by T cells; that production of only the IgG<sub>2a</sub> class allotype (G<sup>2</sup>) is suppressed, presumably as a result of direct interaction between specific T and B cells, and that the capacity of spleen cells to suppress host allotype production can be transferred serially from one C.B-17 mouse to another and as yet without obvious limit.

Mouse 7S Ig allotype determinants of BALB/c (F<sup>19</sup>G<sup>1,6,7,8</sup>H<sup>9,11</sup>), of C.AL-20 a BALB/c congenic strain with the allotype of the AL/N inbred strain (F<sup>19</sup>G<sup>4,6,7,8</sup>H<sup>4</sup>) and of C.B-17 (F<sup>3</sup>G<sup>2</sup>H<sup>9,10</sup>) are denoted here according to the Potter-Lieberman nomenclature where F, G and H refer to the IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub> subclasses, respectively<sup>1</sup>. Anti-allotype immunity was induced in BALB/c, C.AL-20 and C.B-17 donor mice by reciprocal immunisation with each other's Ig in the manner described previously<sup>8</sup>. The recipients in each cell transfer experiment were tested for the presence or absence of different allotypes by means of gel precipitation in micro-Ouchterlony plates.

The kinetics of G<sup>2</sup> suppression in C.B-17 mice that received thymocytes, bone marrow cells or spleen cells from BALB/c mice immune to G<sup>2</sup> are shown in Table 1. After the intravenous injection of  $3 \times 10^6$ - $5 \times 10^6$  thymocytes into X-irradiated C.B-17 recipients (550 r.), G<sup>2</sup> began to disappear as early as 2 weeks later and after 5 weeks was undetectable (groups A and B). Host allotype suppression was chronic and although not shown here, it still persists in C.B-17 recipients 1 yr after cell transfer. The injection of comparable numbers of bone marrow cells (mostly B cells) did not, however, suppress G<sup>2</sup> (groups C and D). Moreover, the exposure of suppressor spleen cells to AKR mouse antiserum specific for T cells (anti- $\theta$  serum) followed by the application of complement (C') abrogated the ability of these cells to suppress G<sup>2</sup> production (groups E and F). Spleen cells treated with anti- $\theta$  serum gave a good proliferative response (<sup>125</sup>I-5'-iododeoxyuridine incorporation) to *E. coli* lipopolysaccharide (Bacto 055-B5), a B-cell mitogen<sup>9</sup>, but unlike the control spleen cells, showed only a marginal proliferative response to phytohaemagglutinin (Gibco), a T-cell mitogen<sup>9</sup> (not shown). These latter results with mitogens were taken as independent evidence for the efficacy and specificity of anti- $\theta$  treatment. We conclude that G<sup>2</sup> suppression is dependent on T-cell immunity.

As Table 2 shows, the antigenic basis for host allotype suppression relates solely to the G<sup>2</sup> globulin. First, the involvement of a non-Ig serum antigen is precluded since spleen cells from BALB/c mice that were made immune to chromatographically pure Fc fragments of C57BL/6 7S

**Table 1** T-cell-dependent suppression of the IgG<sub>2a</sub> allotype (G<sup>2</sup>) in X-irradiated C.B-17 mice after their intravenous injection with BALB/c lymphoid cells immune to G<sup>2</sup>

Group	No. of immune cells injected*		Spleen	No. of recipients showing G <sup>2</sup> /No. injected† (weeks after cell transfer)					
	Thymus	Bone marrow		1	2	3	4	7	20
A	3 × 10 <sup>6</sup>			5/5		5/5	2/5	0/5	0/4
B	5 × 10 <sup>6</sup>			18/18	9/18	3/18	0/18		0/16
C		3 × 10 <sup>6</sup>		6/6		6/6		6/6	6/6
D		5 × 10 <sup>6</sup>		6/6		6/6		6/6	6/6
E			10 × 10 <sup>6</sup>	14/14	2/14	0/14		0/14	
F			10 × 10 <sup>6</sup> (treated with anti-θ serum)	13/13	13/13	13/13		13/13	

\*BALB/c mice were made immune to the IgG<sub>2a</sub> allotype of C57BL mice (G<sup>2</sup>) after five or more intraperitoneal injections of C57BL/6 serum hyperimmune to rabbit red cell ghosts; all BALB/c donors were producing precipitating antibody against G<sup>2</sup> at the time of cell transfer. The AKR mouse anti-θ serum came from Dr J. B. Smith of this institute; exposure of spleen cells to this antiserum was done as follows (group F): 10<sup>7</sup> cells per ml were incubated for 45 min with a 1/16 dilution of antiserum (in phosphate-buffered saline containing 0.1% bovine serum albumin) at 37 °C in a 5% CO<sub>2</sub> atmosphere. After one wash, the cells were resuspended in a 1/10 dilution of guinea pig complement (1 ml of complement per 10<sup>7</sup> cells) and incubated for 30 min more at 37 °C. The cells were then washed three times before being injected into C.B-17 mice. Control spleen suspensions were treated as above except AKR normal mouse serum was substituted for AKR anti-θ serum (group E). The results of groups B, E and F represent two or more separate experiments.

†C.B-17 recipients were X irradiated with 550 r. 1 d before cell transfer in the following conditions: 190 kV; 15 mA; filtration, 1 mm Al and 0.5 mm Cu; dose rate 40–45 r. min<sup>-1</sup>. Recipient sera were reacted with BALB/c anti-G<sup>2</sup> in micro-Ouchterlony plates to test for the presence of G<sup>2</sup> allotype.

Ig were able to suppress G<sup>2</sup> production in C.B-17 mice (group C). Moreover, C.B-17 serum (group B) could substitute for C57BL/6 serum (group A) as an immunogen. C.B-17 serum is not known to be different from that of BALB/c except to contain Ig allotypes of C57BL mice. Second, allotype determinants that characterise the IgG<sub>1</sub> (F<sup>a</sup>) and IgG<sub>2b</sub> (H<sup>a</sup>) subclasses continue to be produced in G<sup>2</sup>-suppressed mice. Evidence of this is deduced from the fact that both anti-F<sup>a</sup>G<sup>2</sup> (groups A–C) and anti-H<sup>a</sup> (group D) gave precipitin bands with serum from G<sup>2</sup>-suppressed C.B-17 mice. It is important to note that the use of C.AL-20 donor mice immune to G<sup>2</sup> was necessary in group D because the IgG<sub>2b</sub> molecules of both BALB/c and C.B-17 mice share the H<sup>a</sup> determinant. With BALB/c mice as donors, it would be impossible to distinguish H<sup>a</sup> production of host cells from that of donor cells. Earlier observations by Jacobson *et al.*<sup>4</sup> are consistent with the above results as Ig-4b (F<sup>a</sup>) was also not suppressed in mice that were suppressed for Ig-1b (G<sup>2</sup>) production by means of neonatal exposure to anti-allotype serum.

From the above, it seems clear that the IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub> subclasses can be produced independently of one another. This could mean that B cells independently differ-

entiate into producers of IgG<sub>1</sub>, IgG<sub>2a</sub> or IgG<sub>2b</sub>, or that C<sub>H</sub> genes for the IgG subclasses are expressed sequentially in differentiating immune cells, in which case the expression of IgG<sub>1</sub> and IgG<sub>2b</sub> must precede that of IgG<sub>2a</sub>. It also seems clear that BALB/c suppressor T cells must have allotype receptors for G<sup>2</sup> (G<sup>2</sup>-suppressor T cells). With G<sup>2</sup> recognition acting as the stimulus, such T cells might proceed to kill G<sup>2</sup>-bearing B cells directly, or alternatively, they might elaborate a suppressive factor which specifically prevents B cells from becoming G<sup>2</sup> producers. A mechanism similar to the latter is perhaps to be favoured in view of recent evidence for T-cell factors that have cooperative<sup>12,13</sup> or suppressive effects<sup>9,14</sup> on specific Ig production.

A more indirect mechanism can be postulated: in this the target of G<sup>2</sup>-suppressor T cells is another T cell that specifically helps B cells produce G<sup>2</sup> (G<sup>2</sup>-helper T cell). This would imply that G<sup>2</sup>-helper T cells carry G<sup>2</sup> determinants on their surface. To suggest any other basis for discrimination seems unwarranted since the specificity of G<sup>2</sup> suppression requires immunity to only the allotype determinants located on the Fc fragment of IgG<sub>2a</sub> molecules (Table 2). We have yet to obtain any evidence for G<sup>2</sup>-helper T cells.

The long term persistence of allotype suppression

**Table 2** Restriction of allotype suppression in X-irradiated C.B-17 mice to the IgG<sub>2a</sub> allotype (G<sup>2</sup>)

Group	Donor mice* (7S Ig allotypes)	Donor mice immunised with†	C.B-17 host allotypes (F <sup>a</sup> G <sup>2</sup> H <sup>a</sup> , <sup>16</sup> ) present 3–5 weeks after intravenous injection of donor spleen cells§ (No. of recipients positive/No. injected)		
			F <sup>a</sup>	G <sup>2</sup>	H <sup>a</sup>
A	BALB/c (F <sup>19</sup> G <sup>1,6,7,8</sup> H <sup>9,11</sup> )	C57BL/6 serum	7/7	0/7	
B	(F <sup>19</sup> G <sup>1,6,7,8</sup> H <sup>9,11</sup> )	C.B-17 serum	8/8	0/8	
C	(F <sup>19</sup> G <sup>1,6,7,8</sup> H <sup>9,11</sup> )	Fc fragment of C57BL/6 7S Ig	9/9	0/9	
D	C.AL-20 (F <sup>19</sup> G <sup>4,6,7,8</sup> H <sup>a</sup> )	C57BL/6 serum		0/8	8/8

\*C.AL-20 donor mice are BALB/c congenic mice that carry the Ig allotype of the AL/N mouse strain. These mice were given by Dr Michael Potter of the National Cancer Institute. F, G and H denote the IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub> subclasses, respectively; the numbers correspond to individual allotypic determinants (nomenclature of Potter and Lieberman<sup>1</sup>). Mouse IgG<sub>1</sub>, IgG<sub>2a</sub> and IgG<sub>2b</sub> have been alternately denoted by Herzenberg *et al.*<sup>2</sup> as Ig-4, Ig-1 and Ig-3, respectively. Here a lower case letter is used to distinguish allotypes (for example, Ig-1a and Ig-1b) and a second number is used to designate individual allotype determinants (for example, Ig-1.1).

†Donor mice were immunised as described in Table 1 except that the first antigen injection was given in Freund's complete adjuvant. Pápain digestion<sup>10</sup> of C57BL/6 7S Ig gave Fc fragments that were purified over DEAE-cellulose according to the method of Fahey and Askonas<sup>11</sup>.

§C.B-17 recipients were X irradiated with 550 r. 1 d before cell transfer in the conditions described in Table 1. Each recipient was injected intravenously with 20 × 10<sup>6</sup> spleen cells from the respective donor mice. The antisera used to test for the presence of F<sup>a</sup>, G<sup>2</sup> and H<sup>a</sup> in C.B-17 recipients were C.B-17 anti-F<sup>a</sup>G<sup>2</sup>, BALB/c anti-G<sup>2</sup> and C.AL-20 anti-H<sup>a</sup>. The indicated specificity of these antisera was assured after testing them against the Igs of various plasmacytomas which were grown in BALB/c, C.B-17 or C.AL-20 mice.

prompted us to transfer serially the spleens of G<sup>2</sup>-suppressed recipients into other C.B-17 mice. In this way, we intended to evaluate the potential for expansion of T cell-mediated immunity. Of three such experiments attempted, Table 3 shows the results of the one that has been under way for the longest time. Starting with the entire spleen of one BALB/c mouse immune to G<sup>2</sup>, we have suppressed G<sup>2</sup> production in 78 C.B-17 recipients. This corresponds to 91% of all injected recipients and covers four cell transfer generations and a cumulative time span of 46 weeks.

general context, it is interesting to speculate that allotype-specific suppressor T cells may constitute part of a normal regulatory system which controls the production of different Ig allotypes by B cells.

As far as we know this is the first time that chronic allotype suppression has been initiated in adult mice and in mice with a genetic background different from SJL. The abnormalities of Ig regulation in SJL mice<sup>18</sup> have been considered as a possible explanation for the susceptibility of (SJL×BALB/c) F<sub>1</sub> mice to chronic allotype suppression.

Table 3 Propagation of IgG<sub>2a</sub> allotype (G<sup>2</sup>) suppression in X-irradiated C.B-17 mice by serial transfer of spleen cells

Group	Cell transfer generation	Cumulative time (weeks)	No. of donors*	No. of C.B-17 mice used as recipients†	No. of recipients showing G <sup>2</sup> /No. injected (weeks after cell transfer)				
					2	3	4	5	6
A	1			6	0/6	0/6			0/6
B	2	9	3	18	18/18	10/18	1/18	0/17§	0/16
C	3	28	2	6		6/6	6/6	0/5§	0/5
				6TX		6/6	5/6	5/6	1/6
D	3	35	3	10		10/10	2/10	1/10	0/9§
				10TX		10/10	7/10	6/10	1/10
E	3	36	4†	24	17/24	6/24	1/23§		0/22§
F	4	40	1	6		6/6	2/6		0/6

TX, thymectomised.

\*The suppression of G<sup>2</sup> in C.B-17 mice was initiated with the spleen of one BALB/c mouse immune to G<sup>2</sup>; the spleen was divided equally as a cell suspension among six C.B-17 recipients (group A). When G<sup>2</sup> suppression occurred, three of these C.B-17 recipients were used as spleen cell donors for serial transfer into other C.B-17 mice. This kind of procedure was repeated over four cell transfer generations, as indicated. All cell transfers were done intravenously.

†Four of the G<sup>2</sup>-suppressed mice in group D (non-TX recipients) were immunised exogenously with six injections of C57BL/6 serum before being used as donors. Normal, unirradiated C.B-17 mice served as controls for the hyperimmunisation. These did not produce precipitating antibody to G<sup>2</sup>, neither did the transfer of their spleens into other C.B-17 mice cause G<sup>2</sup> suppression.

‡All C.B-17 recipients received 550 r. of X irradiation 1 d before cell transfer. Half of the C.B-17 mice in groups C and D were thymectomised 4 weeks before serving as recipients for cell transfer. Recipient sera were reacted with BALB/c anti-G<sup>2</sup> sera in micro-Ouchterlony plates to test for the presence or absence of G<sup>2</sup>.

§Times at which one or more of the injected recipients were found dead.

Two further points should be made: (1) Exogenous immunisation of G<sup>2</sup>-suppressed recipients with hyperimmune serum of C57BL/6 mice boosted the potential of these recipients to suppress G<sup>2</sup> production in other C.B-17 mice (group E); this potential was not yet exhausted at the fourth transfer generation (group F). (2) Thymectomy of prospective C.B-17 recipients delayed the onset of G<sup>2</sup> suppression (groups C and D). The reason for this is unclear.

Two explanations for the extended serial transfer of G<sup>2</sup> suppression in C.B-17 recipients seem reasonable. The first is that G<sup>2</sup>-suppressor T cells of the original donor can be clonally propagated—although with this as the sole explanation, it is difficult to understand why B-cell immunity (antibody responses) cannot be expanded as easily as the present example of T-cell immunity<sup>15</sup>. Therefore, a second explanation inclusive of the idea of clonal expansion is that suppressor T cells directed against G<sup>2</sup> are generated in C.B-17 host mice.

Support for the second explanation comes from the demonstrations<sup>4,16</sup> of G<sup>2</sup> allotype suppression in adult (SJL×BALB/c) F<sub>1</sub> mice that earlier were suppressed neonatally by continued exposure to anti-G<sup>2</sup> antibody. About half of the mice so treated were chronically suppressed beyond 6 months of age; the spleen cells of these mice could suppress G<sup>2</sup> production of normal (SJL×BALB/c) F<sub>1</sub> spleen cells both *in vivo* and *in vitro*<sup>5,6</sup>. This was shown to be due to suppressor T cells which survive indefinitely as a population and apparently derive from the (SJL×BALB/c) F<sub>1</sub> hosts.

If the idea of autosuppressor T cells is applied to our results this suggests that the same genes which enable BALB/c T cells to suppress G<sup>2</sup> production are present in C.B-17 mice. This possibility seems reasonable since BALB/c and C.B-17 mice share the same major histocompatibility (H-2) locus and many immune functions of T cells are under the control of immune response genes that map with the H-2 locus (reviewed in ref. 17). In this

sion<sup>4,6</sup>. But this consideration does not obviously apply to our results because BALB/c and C.B-17 mice do not show Ig irregularities like those in SJL mice. Therefore, the use of BALB/c congenic mice seems to provide a model autoimmune system in which to study the long term effects of constant stimulation and suppression of specific immune cells.

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## Decreased resistance in some heterozygotes in H-2-linked immune response to polyoma-induced tumour

THE relationship between the major histocompatibility complex (MHC) and immune response (*I*r) genes has been demonstrated in the case of several antigens, including some transplantation antigens<sup>1</sup>. Among these, some tumour-associated transplantation antigens (TATA) are probably included<sup>1</sup>. To date, however, few results are available to document this prediction. In the mouse quantitative transplantations of chemically-induced neoplasms suggest that the H-2-linked *I*r genes of different F<sub>1</sub> hybrids can control their susceptibility to parent-specific tumours<sup>2</sup>. This control, however, is certainly complex (that is, involving more than one gene), possibly because this sort of tumour can have different types of TATA<sup>3</sup>.

Using a better defined antigen, the X1 leukaemia-specific antigen, Sato *et al.*<sup>4</sup> demonstrated that the success of transplantation immunity, induced in specifically immunised mice, is H-2 dependent. Antitumour resistance is dominantly inherited in those F<sub>1</sub> hybrids which have appropriate *I*r gene(s). In the present studies, we found a clearcut H-2 dependence concerning transplantation immunity against another sort of TATA, those of a polyoma-induced tumour. Also, the recent prediction of Willims *et al.*<sup>3</sup>, that some H-2 (or *I*r genes) combinations would lead to suppression of antitumour responsiveness is substantiated by some of our results.

of the A.SW parental strain (H-2<sup>a</sup>) and semisynthetic F<sub>1</sub> hybrids of the following combinations, obtained with congenic resistant partner strains: (A/Sn × A.SW)F<sub>1</sub> (H-2<sup>a</sup>/H-2<sup>a</sup>), (A.BY × A.SW)F<sub>1</sub> (H-2<sup>b</sup>/H-2<sup>a</sup>) and (A.CA × A.SW)F<sub>1</sub> (H-2<sup>i</sup>/H-2<sup>a</sup>). Theoretically, the only traits by which all these mice differ is one half of the H-2 and closely-linked genetic material. Animals of both sexes were used and equally distributed among the groups.

The results of these transplantation assays were observed as tumour incidences (number of mice dying of the tumour/number of injected mice) and by mean survival times (MST) in days (Tables 1 and 2). They are expressed in Fig. 1 as the mean of 1/MST, taking the value of 0 for mice which rejected the tumour graft. This enables the representation of the susceptibility to the tumour, using the information from two different criteria<sup>5</sup>. As both types of immunisation procedures (two independent experiments having been carried out with each of them) gave superimposable results, all the data are combined in Fig. 1.

It can be seen that immunisation considerably diminished susceptibility in the A.SW and (A/Sn × A.SW)F<sub>1</sub> groups, whereas no protective effect was detected in (A.BY × A.SW)F<sub>1</sub> and (A.CA × A.SW)F<sub>1</sub> groups. This confirms that some F<sub>1</sub> hybrids can be less resistant than the parental strain to a parental strain-specific tumour<sup>7-9</sup>, even after immunisation.

This seems somewhat aberrant because the H-2-linked *I*r genes generally are dominantly expressed for the highest response. Different mechanisms can be proposed for explaining the present finding: (1) A cross immunity between the TATA of SEWA and normal transplantation antigens contained in these hybrids which are not immunised against

**Table 1** Susceptibility of mice challenged with 10<sup>3</sup> viable SEWA cells intraperitoneally, after specific protection by amputation of the tumour-bearing leg (pool of two independent experiments)

Strains	H-2	Treatment	Tumour incidence	MST ± s.e. (d)
A.SW	s/s	Non-treated	9/10	47.3 ± 2.0
		Protected	1/9	71
(A.BY × A.SW)F <sub>1</sub>	b/s	Non-treated	2/2	34.0 ± 2.0
		Protected*	3/3	35.6 ± 2.0
(A/Sn × A.SW)F <sub>1</sub>	a/s	Non-treated	14/16	49.3 ± 5.9
		Protected	1/12	53.0
(A.CA × A.SW)F <sub>1</sub>	f/s	Non-treated	10/10	46.1 ± 1.4
		Protected	3/10	41.0 ± 6.2

\*In the first experiment of protection, all mice in this group died of tumour after the amputation of the tumour-bearing leg, in spite of the fact that they were treated according to the same schedule as the mice of the other groups. This further suggests that the mice of this genome are less resistant. Results for mice in this experiment were excluded from the Table.

The SEWA line used was derived from a polyoma-induced osteosarcoma<sup>3</sup>. It was maintained *in vivo* in A.SW mice by intraperitoneal, consecutive passages as an ascitic tumour. Non-treated and specifically immunised animals were injected. Two modes of immunisation were used: (1) Amputation of a tumour-bearing leg, the site of subcutaneous injection of 4 × 10<sup>4</sup> ascitic SEWA cells 15–25 d previously (Table 1). (2) Three weekly subcutaneous injections of 10<sup>6</sup> plaque-forming units (PFU) polyoma virus (small plaque strain) (Table 2). One to three weeks after completion of immunisation, mice (three months old) received an intraperitoneal challenge of 10<sup>3</sup> viable cells. Mice used were

the SEWA tumour could be envisaged. Such common transplantation specificities between TATA and normal allogeneic antigens have already been reported in the case of chemically-induced tumours<sup>8,10</sup>. (2) Also, one example of dominantly expressed non-response of an H-2-linked *I*r gene has been reported<sup>11</sup>. Thus, allowing a good anti-TATA response the H-2<sup>a</sup>/H-2<sup>a</sup> chromosome of the A.SW could be recessive in the H-2<sup>a</sup>/H-2<sup>b</sup> and H-2<sup>a</sup>/H-2<sup>i</sup> heterozygotes. We do not, however, favour *a priori* such an explanation because all other works reported dominance of the response and not of the non-response. The finding reported in ref. 10 seems to be most exceptional.

**Table 2** Susceptibility of mice challenged with 10<sup>3</sup> viable SEWA cells intraperitoneally, after specific protection by three weekly subcutaneous injections of 10<sup>6</sup> PFU polyoma virus (pool of two independent experiments)

Strains	H-2	Treatment	Tumour incidence	MST ± s.e. (d)
A.SW	s/s	Non-treated	10/15	39.6 ± 3.4
		Protected	0/15	—
(A.BY × A.SW)F <sub>1</sub>	b/s	Non-treated	5/5	37.4 ± 1.2
		Protected	9/11	33.1 ± 3.0
(A/Sn × A.SW)F <sub>1</sub>	a/s	Non-treated	9/10	37.8 ± 2.2
		Protected	0/10	—
(A.CA × A.SW)F <sub>1</sub>	f/s	Non-treated	8/8	48.7 ± 0.8
		Protected	8/10	47.0 ± 2.3

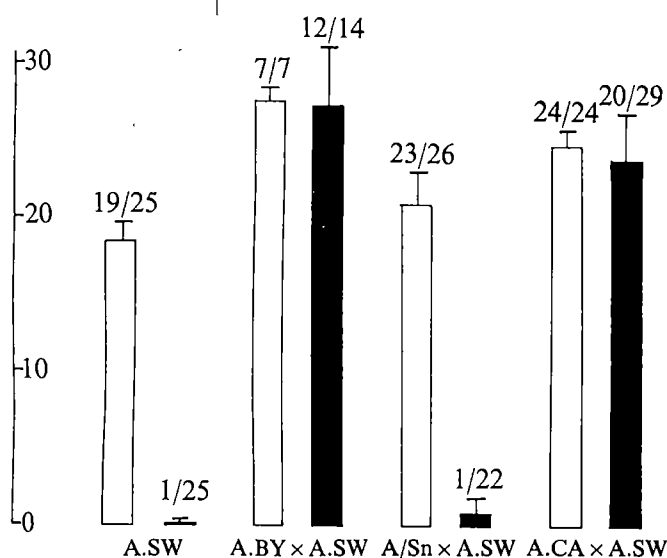


Fig. 1 Susceptibility, expressed as 1/MST (arbitrary units) of A.SW, (A.BY x A.SW) $F_1$ , (A/Sn x A.SW) $F_1$  and (A.CA x A.SW) $F_1$  to  $10^3$  SEWA tumour cells. Open column, non-treated mice; Solid columns, mice immunised by two different procedures; error bars are 1 s.e.; figures above bars, pooled tumour incidence of corresponding experimental groups.

(3) That  $H-2^s/H-2^b$  and  $H-2^s/H-2^d$  are less protected against the tumour graft than  $H-2^s/H-2^s$  could be explained, on the basis of a higher response of the heterozygotes, in two ways: (i) The non-protected  $F_1$  hybrids could have a higher anti-TATA reaction giving rise to the production of enhancing antibodies, as already discussed in another situation by Huemer<sup>7</sup>. Further experiments could clarify this point, by transferring the serum of non-protected immunised hybrids to test animals (ii) Recent findings led Prehn to hypothesise that a mild immune reaction, in certain cases, could enhance tumour growth instead of suppressing it<sup>12</sup>. Although this sort of mechanism is difficult to prove in the present examples, it may explain our results.

In conclusion, and whatever the exact mechanism, the results demonstrate that one half of the H-2 and H-2-associated genetic material may detectably influence the intensity of the anti-TATA immune rejection of a polyoma-induced tumour.

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## Sprouting of synapses after partial denervation of frog cardiac ganglion

SYNAPTIC connections in the adult nervous system of several species can be quite flexible and this characteristic has been termed neuronal plasticity. For example, when lesions are made in the axonal pathways of the brains of mature rats, surviving nerve terminals can proliferate and innervate the synaptic sites vacated by the degenerating axons<sup>1,2</sup>. This "sprouting" occurs in the central nervous system<sup>3-4</sup>, autonomic nervous system<sup>5</sup> and peripheral nervous system<sup>6</sup>, and seems to be a characteristic response of nervous tissue to partial denervation. In only a few studies has sprouting been reported not to occur<sup>7-9</sup>. Sprouting of synapses has important implications for the maintenance of neuronal connections and the ability of the nervous system to recover from focal lesions. But several questions need to be answered. For example, are sprouted synapses functional, and if so, do they transmit normally? Is there any specificity in or control over the proliferation of new connections? What initiates and terminates the sprouting? We can now begin to answer these questions. Our studies on the parasympathetic cardiac ganglion of the frog have shown that synaptic sprouting occurs very rapidly after partial denervation. Furthermore, the number of boutons per cell remains constant throughout sprouting and is the same as the number per cell in control ganglia.

The cardiac ganglion in the frog, which lies in the thin, interatrial septum<sup>10</sup>, was dissected from the heart and kept in a shallow recording chamber in frog Ringer (112 mM NaCl, 2 mM KCl, 3 mM HEPES buffer (pH 7.2) and 5 mM  $CaCl_2$ ). The calcium concentration was increased slightly to enhance synaptic transmission<sup>12</sup>. The preganglionic (vagus) nerves were drawn into separate suction electrodes to stimulate right or left vagus nerves independently. Ganglion cells on the left branch of the septum (Fig. 1) were impaled

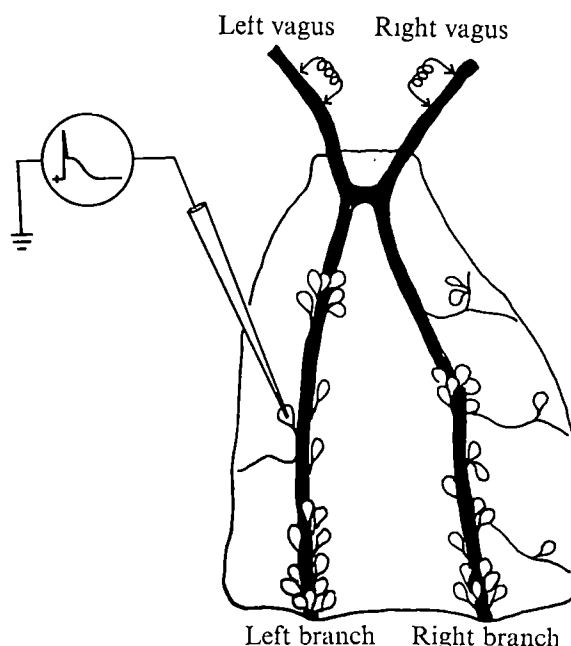
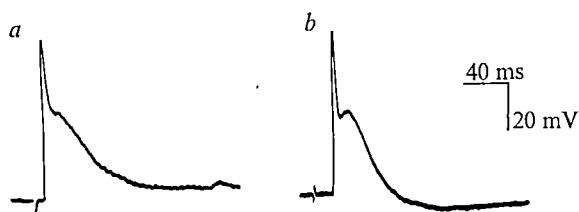


Fig. 1 Schematic drawing of the interatrial septum in the frog showing the arrangement of stimulating and recording electrodes. The cardiac ganglion cells are scattered along branches of the vagus nerves which traverse the septum. Near the entry to the heart many axons cross between left and right branches. The left and right vagus nerves to the heart were drawn into separate suction electrodes. Ganglion cells along the left vagal branch were impaled and their synaptic input from either (both) vagus nerve(s) was mapped.

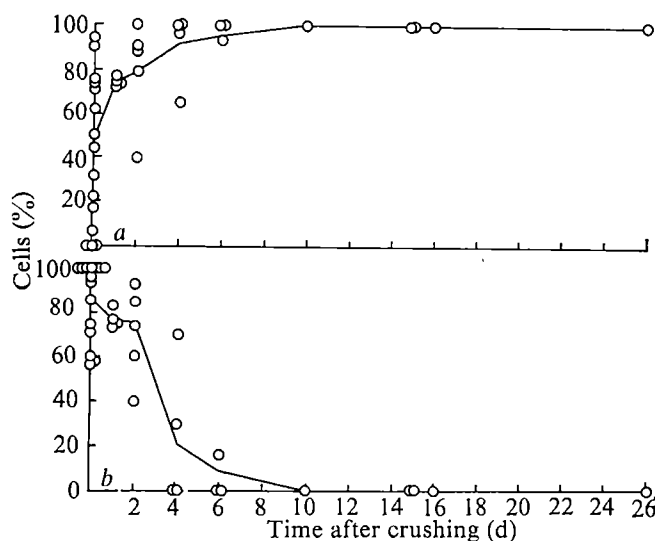


**Fig. 2** Intracellular recordings of responses from ganglion cells, evoked by stimulation of the right vagus nerve in a normal ganglion (*a*) and in one in which the right vagus nerve had sprouted to innervate the entire ganglion (*b*). In both instances a large excitatory postsynaptic potential evoked an impulse and outlasted the action potential. In (*b*), the left vagus nerve had been crushed 16 d previously; the left vagus axons had degenerated and the right vagus endings had proliferated to contact all the ganglion cells.

with high resistance micropipettes and excitatory post-synaptic responses to right and left vagal stimulation were photographed. In some frogs, the left vagus nerve was exposed near its exit from the skull and crushed while the animals were anaesthetised with 0.5% tricaine methanesulphonate (Sigma). These operated animals were kept at room temperature in running tap water.

To study the sprouting of synaptic connections in the ganglion after partial denervation, we mapped the vagal innervation of cells on the left branch in normal frogs and operated frogs. In normal frogs, each ganglion cell is usually innervated by only one or sometimes two vagal fibres<sup>2</sup>, and, as might be expected, neurones tend to be innervated by the ipsilateral vagus nerve. Eighty-seven per cent of the cells on the left branch had input(s) from the left vagus nerve and 48% had input(s) from the right vagus nerve (207 cells from 15 ganglia). A small proportion of these cells (34%) had inputs from both nerves. An even smaller fraction (3%) had no inputs.

When the vagal innervation of neurones on the left branch was mapped in the operated frogs, nearly all the cells received input(s) from the intact (right) vagus nerve.



**Fig. 3** Sprouting of preganglionic nerve fibres to the parasympathetic cardiac ganglion in the frog; increase in the percentage of ganglion cells innervated by the right vagus nerve after the left vagus nerve had been crushed *a*, Percentage of neurones from the left branch of the cardiac ganglion which received synaptic input from the contralateral vagus nerve on various days after crushing the ipsilateral vagus nerve. *b*, Percentage of neurones which received synaptic input from the left vagus nerve after the operation. Day zero shows data from 15 unoperated ganglia. Each symbol represents data from one ganglion (20–30 cells impaled in each ganglion) and a solid line has been drawn through the weighted means. Percentages were calculated on the basis of the total number of cells which received any synaptic input.

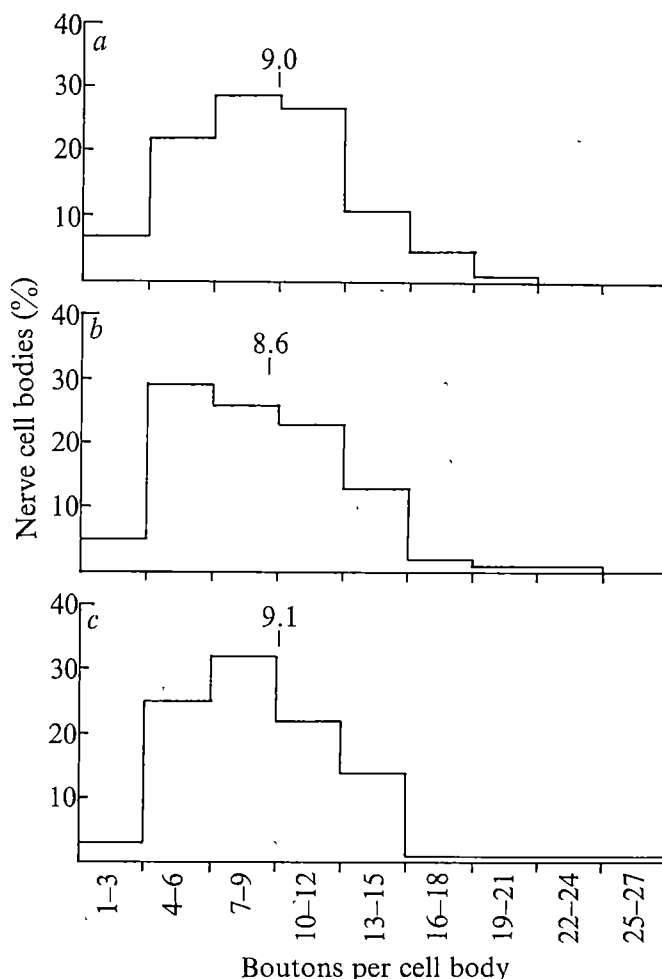
For example, 10–26 d after the left vagus nerve was crushed, 100% of cells with synaptic inputs was innervated by the right vagus nerve (compare 48% for unoperated frogs). Six per cent had no inputs; this latter figure is similar to that in normal animals and presumably due to damage caused during dissection. Because there was no visible loss of ganglion cells after the operation, we interpret this increased percentage of contralateral innervation as due to a proliferation, or sprouting, of the surviving right vagus nerve.

The “sprouted synapses” are functional, and as far as we could detect, transmit normally (Fig. 2). No abnormal synaptic responses were evoked during or after sprouting. Experiments are in progress to examine synaptic transmission in greater detail to determine whether we can resolve any peculiarities of sprouting synapses.

The time course of sprouting was examined by mapping vagal innervation of different ganglia at several intervals after the operation. Figure 3 shows that as soon as 24 h after crushing of the left vagus nerve, there was a small but significant ( $P < 0.01$ ) increase in innervation by the right vagus nerve. The maximum extent of sprouting was reached about 8 days after the operation; there was no further increase in the proportion of cells innervated by the right vagus nerve.

To study the morphological basis of this phenomenon

**Fig. 4** Histograms of numbers of synapses per cell body before (*c*), during (*b*), and after (*a*) sprouting of preganglionic inputs. Synaptic boutons were counted in whole mounts of ganglia stained with zinc iodide. In *c*, boutons from 73 neurones on the left branches in nine unoperated ganglia were counted. In *b*, boutons were counted from 112 ganglion cells (left branch) from animals in which the left vagus nerve had been crushed 1–8 d previously. In *a*, boutons were counted from 120 neurones (left branch) from animals 9–26 d after the operation. Numbers above each histogram are the mean values.



and determine whether we could correlate the changes in innervation as determined physiologically with any structural changes, we stained synaptic boutons in whole-mount preparations with the zinc-iodide method<sup>10,11</sup> for light microscopy. Although the basis of the zinc iodide method is not well understood, we assume that there is no difference in the ability of sprouted synapses and normal synapses to take up the stain. In normal frogs, the average number of synaptic boutons per cell on the left branch was  $9.1 \pm 4.3$ . When boutons were counted in ganglia after the left vagus nerve had been crushed, and sprouting of the right vagus nerve was complete, there was no change ( $9.0 \pm 3.9$  boutons per cell). Boutons were counted at several intervals after crushing of the left vagus nerve. Figure 4 summarises the measurements and shows that there was no change in the number of boutons per cell during sprouting (1–8 d after the operation) or after its completion (9–26 d after the operation).

These findings indicate that surviving vagal axons respond rapidly to partial denervation of the ganglion by sprouting and form new functional synapses. Furthermore, the morphological studies show that sprouting is a well controlled phenomenon. Thus, the proliferation of synapses does not change the number of boutons from that found on normal cells. These findings are consistent with the hypothesis that sprouting synapses grow to vacant synaptic sites<sup>2</sup> and that each cell has a fixed number of these sites<sup>7</sup>.

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## Molecular differentiation in the preimplantation mouse embryo

THE primary observable event of differentiation in the mammalian embryo is the formation of the trophectoderm and the inner cell mass (ICM). Differentiation first becomes evident in the mouse embryo during early blastulation and is clear and apparently irreversible by the 60-cell blastocyst stage. The 45 or so trophectoderm cells surround a blastocoelic cavity containing the ICM, they pump fluid, are phagocytic, are interconnected through junctional complexes and can elicit an implantation response in the uterus<sup>1</sup>. The trophectoderm gives rise to the ectoplacental trophoblast, primary and secondary trophoblastic giant cells and probably also to extra-embryonic ectoderm<sup>1,2</sup>. The cells of the ICM are less evidently differentiated, being rounded, sticky cells somewhat like cleavage stage blastomeres, but having lost the capacity to form trophectoderm<sup>3,4</sup>. The ICM gives rise to embryonic ectoderm, and to both extra-embryonic and embryonic endoderm<sup>1,2,5</sup>. Although

the fully differentiated cells of the blastocyst are distinguishable by structural and functional criteria, adequate markers of differentiation are not available for the morula stage, when the determinative events leading to differentiation are presumed to occur. Thus, attempts to detect blastomeres with a commitment to one or the other differentiated cell type have relied for an index of commitment on the subsequent behaviour of genetically or physically marked blastomeres combined in various spatial relations with other distinguishable blastomeres. This experimental approach is open to criticism. Frequently, many cell divisions must elapse before suitable genetic markers can be detected with sufficient sensitivity, and the procedures involve artificial manipulation and therefore possibly modification of blastomeres to test their potency<sup>2,6</sup>. The detection of a cell marker which is produced *in situ* in the earliest stages of differentiation, and one which is detectable by highly sensitive techniques would circumvent both of these problems. We describe here the detection of tissue specific markers which may prove to fulfil these criteria.

Mouse blastocysts were flushed from the uteri of super-ovulated mice of an inbred strain carrying the *a<sup>c</sup>* gene (extreme non-agouti) which had been mated to *a<sup>c</sup>/a<sup>c</sup>* males 3.5 d previously (detection of vaginal plug indicated day 0). All blastocysts had recently expanded, with zona pellucida intact and with no evidence of trophoblastic giant cell formation. Blastocysts from several females were pooled for each experiment and then divided into three groups: the first group was left intact, the second group was used for microdissection of ICMs and the third for microdissection of trophoblastic vesicles<sup>1</sup>. Dissection of the trophoblastic vesicles involves loss of the polar trophoblast overlying the ICM. Occasionally both trophoblastic vesicles and ICMs were recovered from the same blastocysts. Microdissected fragments were incubated in medium PB1+10% foetal calf serum for 2–3 h, and then checked to determine that only cells in the trophoblastic preparation and not those in the ICM preparation were accumulating fluid. This procedure served to test for any major cross contamination of cell type. The embryos and embryonic fragments were then washed in protein-free medium 16 (ref. 7), and transferred to 100  $\mu$ l of medium 16 containing 10  $\mu$ l of <sup>35</sup>S-L-methionine (New England Nuclear, specific activity 200 Ci mmol<sup>-1</sup>). After incubation for 4 h at 37 °C in a moist atmosphere of 5% CO<sub>2</sub> in air, the blastocysts and fragments were collected into 25  $\mu$ l of lysis buffer containing urea, 2-mercaptoethanol, Ampholines (LKB, pH 5–7 and 3–10) and non-ionic detergent Nonidet P-40.

It was confirmed that the radiolabelled amino acid had been incorporated into proteins by measuring trichloroacetic acid-precipitable counts of aliquots of each sample<sup>8</sup>. Microdissection of embryos did not seem in most experiments to reduce the ability of fragments to incorporate label, when allowance was made for the loss of approximately one third of embryonic tissue during dissection. The relative rate of protein synthesis was comparable with that previously observed in intact blastocysts at the same stage of development<sup>10</sup>. In each of six experiments, a minimum of 8–10 microdissected fragments of each tissue was used.

Labelled proteins were separated by two-dimensional polyacrylamide gel electrophoresis<sup>9</sup>. After separation of proteins by isoelectric focusing on cylindrical gels, proteins were electrophoresed into exponential (8–15%) gradient, sodium dodecyl sulphate (SDS)–polyacrylamide slab gels<sup>8,9</sup>. After electrophoresis, the slab gels were infiltrated with the scintillant PPO<sup>11</sup>, dried under vacuum, placed under X-ray film (Kodak Royal-X-Omat, RP/R54) and stored at –70 °C for 6–10 weeks before developing. Each spot detected on the autoradiographs represented one polypeptide into which label had been incorporated.



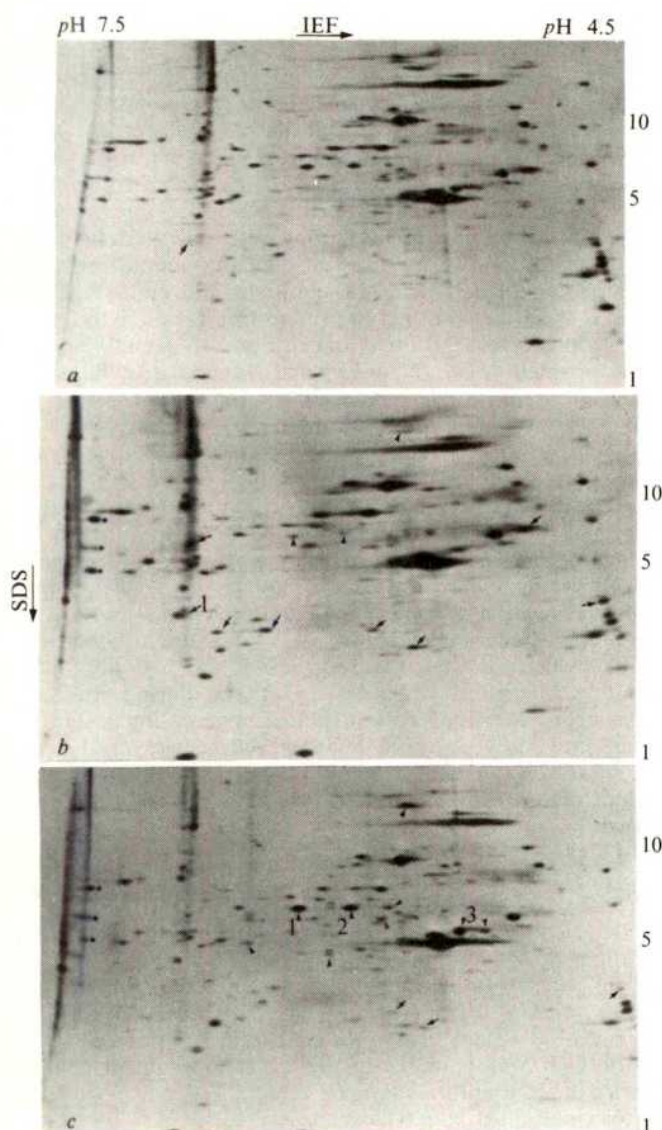
Representative two-dimensional protein patterns of intact blastocysts (*a*), cells of the ICM (*b*) and cells of the trophoblast (*c*) are shown in Fig. 1. Patterns were compared for protein spots common to all three preparations (intact embryo, Fig. 1*a*), spots limited to the ICM and intact embryo (indicated by arrows, Fig. 1*b*) and spots limited to the trophoblast and intact embryo (indicated by solid triangles, Fig. 1*c*). No spots were found exclusively in intact embryos. Most polypeptide spots were common to all three types of preparations, as might be expected since they presumably represent proteins required for general cell function. Several 'tissue-specific' spots, however, were seen consistently. The term 'tissue-specific' spots describes those polypeptides which were either not detectable or only very weakly so in one dissected tissue but clearly present in the other. An occasional faint trace of some of the stronger trophoblast-specific spots was detected on some gels of the ICM (Fig. 1*b*), and vice versa (Fig. 1*c*). Such spots might represent a genuine observation but may alternatively be due to cross contamination by a few cells, made visible by the sensitivity of the technique.

Apart from many apparent quantitative differences between individual spots in the patterns obtained from dissected ICM and trophoblast, ICM tissue yielded at least eight consistent tissue-specific spots (arrows, Fig. 1*b*), while trophoblast tissue yielded a number of obvious, major

'tissue-specific' spots (Fig. 1*c*) of which three spots constituted a relatively substantial fraction of the total incorporated material in the tissue (Fig. 1*c*). In addition, at least three other spots were found in ICM dissections but occurred in either of two different positions relative to all other spots in different experiments (asterisks, left of *b* and *c*). One possible explanation for this observation is that at least three polypeptides differ in the tissues from different dissections. Such a difference could result from genetic heterogeneity in the *a*<sup>0</sup> strain blastocyst or perhaps from the chemical modification of polypeptides, such as the addition or deletion of a carbohydrate group. The chemical modification of polypeptides may be a normal feature of development and could be related to the age of the blastocysts or the position of cells within the embryo. Nine other spots (not marked) were detected in the trophoblast only or in the ICM only but were not consistently observed in the microdissections. These polypeptides were only weakly radioactive and may be more clearly defined with prolonged exposures or with preflashing of the X-ray film<sup>11</sup>.

The failure to detect polypeptide spots unique to intact blastocysts implies that the polar trophoblast overlying the ICM probably does not synthesise a unique set of proteins. One spot which was, however, very weak in the gels of intact embryos (Fig. 1*a*) was relatively intense in gels of ICM (arrow 1, Fig. 1*b*) and absent from the gels of the trophoblast (Fig. 1*c*). This observation could be explained by an ICM-trophoblast interaction, in which quantitative rather than qualitative regulation of protein synthesis was involved. In this case interactions between ICM and trophoblast may have functioned to limit rather than induce the synthesis of a particular polypeptide.

These results have identified molecular correlates of the earlier visible events of differentiation in the mouse. The existence of differences at the molecular level is not surprising in view of the overt differentiation at the cellular level<sup>12</sup>. Indeed, the fact that only a relatively minor proportion of the spots identified are 'tissue-specific' might be considered surprising. But we are not detecting all newly synthesised proteins with this technique, since the limits between which both isoelectric focusing and gradient electrophoresis function will exclude many proteins. Other polypeptides will be only weakly radioactive and will require longer exposure to preflashed X-ray film for detection. In addition, some autoradiographic spots may not necessarily represent the direct expression of new gene products but rather, as a normal feature of development,



**Fig. 1** *a*, Separation of protein labelled with <sup>35</sup>S-L-methionine from intact, 3.5-d *a*<sup>0</sup> blastocysts by high resolution, two-dimensional gel electrophoresis. In the first dimension, proteins were separated according to charge by isoelectric focusing (IEF) on 135-mm cylindrical gels. In the second dimension, proteins were separated according to molecular weight on sodium dodecyl sulphate (SDS), exponential-gradient (8–15% acrylamide), polyacrylamide slab gels. For the first dimension, the pH range was determined by cutting a one-dimensional gel (after isoelectric focusing) into 3-mm pieces, mashing the pieces in degassed, distilled water (2 ml), shaking the mixture for approximately 1 h (in tightly sealed vials) and then measuring the pH of the mixtures. Approximate molecular weights ( $\times 10^{-3}$ ) are given on the far right and were determined from gels calibrated for molecular weight as before<sup>9</sup>. The arrow points to a polypeptide which was absent from trophoblast preparations, relatively intense in ICM preparations (arrow, 1, Fig. 1*b*) but only weakly radioactive in the intact embryo. *b*, Representative autoradiograph of protein labelled with <sup>35</sup>S-L-methionine from ICM dissection. Spots limited to this tissue are indicated by arrows. Faint traces of major trophoblast spots are indicated by solid triangles. Approximate molecular weights ( $\times 10^{-3}$ ) are given on the far right. *c*, Representative autoradiograph of protein labelled with <sup>35</sup>S-L-methionine derived from trophoblast dissection. Spots limited to this tissue are indicated by solid triangles with major spots indicated by triangles 1–3. Faint traces of major ICM spots are marked by arrows. Approximate molecular weights ( $\times 10^{-3}$ ) are given on the far right.



the chemical or enzymatic modification of proteins, with a resulting alteration in spot position. Further study is required to define those spots representing new gene products and those spots representing polypeptides which have been modified after translation.

With the above caveats in mind, the detection of the differences described here presents a powerful approach for the analysis of progressively earlier stages of mammalian development to determine the time at which the 'tissue-specific' proteins first appear and the spatial distribution of the cells producing them. With such an approach using intact, non-manipulated and unmarked embryos, it may prove possible to test directly the hypothesis that cell position determines selective gene expression and thus differentiation in preimplantation morulae<sup>13-15</sup>.

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## Increased cyclic nucleotide phosphodiesterase activity in leukaemic lymphocytes

THE intracellular concentration of cyclic AMP influences cellular proliferation and maturation in both normal and malignant tissue. For example, the concentration of cyclic AMP—changes during the cell cycle being lowest during mitosis<sup>1</sup>—is inversely related to the rate of cell growth<sup>2</sup>, and is elevated in contact-inhibited cell populations<sup>3,4</sup>. In studies of malignant tissue, the concentration of cyclic AMP is lower in cells transformed *in vitro* by oncogenic viruses than in untransformed cells<sup>3-5</sup>, and is lower in certain tumours grown *in vivo* than in the corresponding normal tissue<sup>6,7</sup>. Furthermore, in malignant cells, exogenous cyclic AMP or agents that increase the intracellular concentration of this cyclic nucleotide decrease the rate of growth<sup>8</sup> and induce morphological and biochemical differentiation<sup>9,10</sup>.

The role of cyclic GMP in cell growth is less clear although it has been demonstrated that its concentration increases in lymphocytes responding to plant mitogens<sup>11</sup> and decreases in fibroblasts transformed by oncogenic viruses<sup>12</sup>.

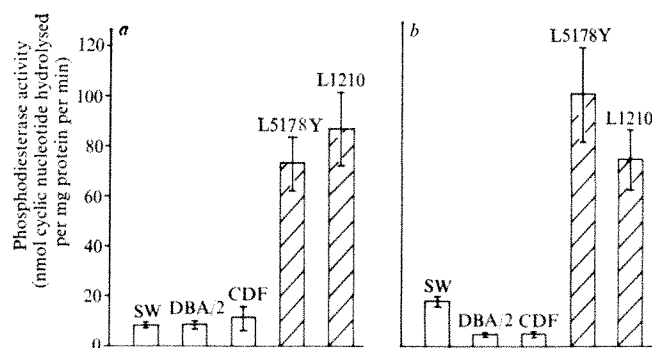
A decreased concentration of cyclic AMP in malignant cells may be caused by a reduction in the activity of the

enzyme catalysing its synthesis (adenylate cyclase) or an increase in the activity of the enzyme catalysing its hydrolysis (phosphodiesterase). There is evidence that both these enzymes are altered in certain transformed cells. For example, BHK cells<sup>13</sup> and astrocytoma cells<sup>14</sup> transformed by oncogenic viruses have lower activities of adenylate cyclase than do untransformed cells. Other types of transformed cells and certain solid tumours did not, however, always exhibit lower activities of adenylate cyclase (for reviews see refs 15 and 16).

Most studies of phosphodiesterase showed that enzyme activity was lower in malignant cells than in normal cells (for review see ref. 17). Other studies showed no change in phosphodiesterase activity in malignant cells<sup>18</sup>, and still others<sup>19</sup> have shown that although hepatomas have a decreased total activity of phosphodiesterase, the activity of the low  $K_m$  form of the enzyme was increased.

We now report that certain leukaemic lymphocytes have markedly increased activities of both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase compared with those of normal lymphocytes. This increased capacity to hydrolyse the cyclic nucleotides may provide an explanation for the abnormal growth of these cells.

Female mice (20-25 g, Jackson Laboratories, Bar Harbor, Maine) were given free access to food and water. Lymphocytic leukaemia cell lines, L5178Y and L1210 (Little, Cambridge, Massachusetts) were grown in the ascites fluid of the peritoneal cavity of C<sub>3</sub>H<sub>2</sub>F<sub>1</sub> mice. Activities of cyclic AMP



**Fig. 1** Cyclic nucleotide phosphodiesterase activity of normal and leukaemic lymphocytes. Normal lymphocytes (open bars) were prepared from the lymph nodes of three strains of mice: DBA/2, the strain in which the tumours originated; C<sub>3</sub>H<sub>2</sub>F<sub>1</sub> (CDF), the strain that serves as host for the tumour; and Swiss Webster (SW), a widely used inbred strain. Lymph nodes were minced in Krebs-Henseleit buffer, pH 8.0. Cells were freed from the stroma in a Dull No. 24 homogeniser using a loose fitting Teflon pestle. Cell suspensions were purified by passage through a plastic column fitted with 40 × 7 mm of loosely-packed siliconised glass wool. Cells were washed twice in buffer and collected by centrifuging at 480g for 10 min. Cells were sonicated for 15 s at 100 W in 50 mM glycylglycine buffer, pH 8.0. Leukaemic lymphocytes, L5178Y and L1210 (hatched bars), were collected 6 d after transplantation into CDF mice by injecting 2 ml Krebs-Henseleit buffer intraperitoneally and aspirating the cell suspension. Cells were prepared in the same manner as were the normal lymphocytes. Using these techniques, both preparations contained more than 95% lymphocytes; 99% of the cells were viable as evidenced by their ability to exclude Trypan blue. Cells were sonicated for 15 s, at 100 W, which was long enough to disrupt more than 90% of the cells without appreciable loss of phosphodiesterase activity. Cyclic nucleotide phosphodiesterase was measured in the whole sonicate using either cyclic AMP (250 μM) or cyclic GMP (100 μM) as substrate. Each tube contained a 50 mM glycylglycine buffer (pH 8.0) containing 0.1 mM CaCl<sub>2</sub> and an enzyme cycling system described in detail elsewhere<sup>19,20</sup>. Protein was measured by the method of Lowry *et al.*<sup>28</sup>. Activity of both cyclic nucleotide phosphodiesterases (on a protein basis) was significantly greater in the leukaemic lymphocytes than in the normal lymphocytes ( $P < 0.005$ ). When the activity was based on the number of cells, the difference in activity between the normal and leukaemic cells was two to three times greater than when the activity was based on protein. Bars represent mean ± s.e. of at least five enzyme preparations. *a*, Cyclic AMP phosphodiesterase; *b*, Cyclic GMP phosphodiesterase.

**Table 1** Kinetic analysis of cyclic nucleotide phosphodiesterases of normal and leukaemic lymphocytes

Cell Source	Cyclic AMP phosphodiesterase		Cyclic GMP phosphodiesterase	
	$V_{\max}$	$K_m$ ( $\mu\text{M}$ )	$V_{\max}$	$K_m$ ( $\mu\text{M}$ )
Normal				
Swiss Webster	11 $\pm$ 2	85 $\pm$ 12	45 $\pm$ 5	38 $\pm$ 5
DBA/2	11 $\pm$ 1	67 $\pm$ 7	7.6 $\pm$ 1.6	40 $\pm$ 4
C <sub>3</sub> D <sub>2</sub> F <sub>1</sub>	22 $\pm$ 6	102 $\pm$ 14	9.6 $\pm$ 1.2	32 $\pm$ 5
Leukaemic				
L1210	224 $\pm$ 20	159 $\pm$ 29	124 $\pm$ 28	60 $\pm$ 7
L5178Y	141 $\pm$ 31	113 $\pm$ 18	225 $\pm$ 48	52 $\pm$ 9

Normal and leukaemic lymphocytes were prepared as in Fig. 1. Cells were sonicated and centrifuged at 100,000*g* for 60 min. The activity of cyclic AMP and cyclic GMP phosphodiesterase was measured in the supernatant fraction.  $V_{\max}$  and  $K_m$  were calculated according to the procedure of Hofstee<sup>30</sup> at substrate concentrations from 15–250  $\mu\text{M}$  cyclic AMP and 12.5–100  $\mu\text{M}$  cyclic GMP.  $V_{\max}$  for both cyclic nucleotide phosphodiesterases was significantly greater in the leukaemic cell preparations than in any of the normal cell preparations ( $P < 0.005$  for all preparations except for Swiss Webster cyclic GMP phosphodiesterase where  $P < 0.01$ ). Each value represents the mean  $\pm$  s.e. for three to five enzyme preparations. Enzyme activity is expressed as nmol cyclic nucleotide hydrolysed per mg protein per min.

phosphodiesterase<sup>19</sup> and cyclic GMP phosphodiesterase<sup>20</sup> were determined as described previously. The rate of hydrolysis of the cyclic nucleotides in these assays was linear with respect to the time of incubation and concentration of tissue. The effect of tissue on the standard curves was determined and, if necessary, appropriate corrections were made. All experiments were designed to study the enzymes from freshly prepared normal and leukaemic lymphocyte preparations on the same day in identical assay conditions.

Figure 1 compares the specific activities of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase in whole sonicates of leukaemic and normal lymphocytes. As can be seen, the phosphodiesterase activity in the leukaemic lymphocytes was 5–10-fold greater than that of any of the other three normal lymphocyte preparations studied. To determine if this increase in phosphodiesterase activity of the leukaemic cell preparations was confined to a specific subcellular element in these cells, we studied the phosphodiesterase activity in various subcellular fractions as well as in the 100,000*g* supernatant fluid. Leukaemic cells exhibited a greater phosphodiesterase activity in all subcellular fractions studied.

A kinetic analysis of the 100,000*g* supernatant fraction of the lymphocyte preparations is shown in Table 1. The activity of phosphodiesterase was determined at substrate concentrations from 1 to 250  $\mu\text{M}$  for cyclic AMP and from 0.8 to 100  $\mu\text{M}$  for cyclic GMP. At all substrate concentrations, the phosphodiesterase from leukaemic cells hydrolysed cyclic AMP and cyclic GMP at a greater rate than did the phosphodiesterase of normal lymphocytes. The calculated maximum velocities ( $V_{\max}$ ) for both the cyclic nucleotide phosphodiesterases from either the L1210 or L5178Y leukaemic lymphocytes was markedly greater than the  $V_{\max}$  of any normal lymphocyte preparation.

This increased reaction velocity could not be attributed

to an increased apparent affinity of the enzyme from the leukaemic cell for its substrate since preparations from normal lymphocytes showed similar or even lower apparent Michaelis constants ( $K_m$  values) than did the preparations from the leukaemic cell lines. At concentrations of cyclic AMP from 15 to 250  $\mu\text{M}$  or at concentrations of cyclic GMP from 12.5 to 100  $\mu\text{M}$ , each phosphodiesterase exhibited a single  $K_m$  value. At lower substrate concentrations, complex kinetic behaviour made accurate assessment of other  $K_m$  values impractical. The complex kinetics observed may indicate the presence of a mixture of isoenzymes in these cells as has been found in most tissues<sup>21,22</sup> including single cell lines<sup>23</sup>.

Several possibilities may account for the observed increase in phosphodiesterase activity of leukaemic lymphocytes. For example, the difference may be attributable to the different environments in which the cells were grown, as the leukaemic cells were in suspension in the ascites fluid of the peritoneal cavity, whereas the normal lymphocytes were in a solid tissue. To examine this possibility we injected leukaemic lymphocytes subcutaneously into CDF mice. Six days later the solid nodule was dissected, and the cells were freed from stroma by the procedure described for the preparation of normal lymphocytes (see Fig. 1). The phosphodiesterase activity of these cells (73  $\pm$  4 nmol cyclic AMP and 65  $\pm$  5 nmol cyclic GMP hydrolysed per mg protein per min) was not significantly different from that of leukaemic cells grown for 6 d in the ascites fluid of the peritoneal cavity of another group of CDF mice (86  $\pm$  5 nmol cyclic AMP and 59  $\pm$  3 nmol cyclic GMP hydrolysed per mg protein per min) indicating that the greater phosphodiesterase activity in leukaemic lymphocytes was probably not caused by the physical environment of the cells.

We next examined the possibility that the increased activity of phosphodiesterase in leukaemic cells could be attributed to the presence in these cells of a heat-stable, calcium-dependent, protein activator of phosphodiesterase which has been found previously in almost all other tissues<sup>24,25</sup>. We assayed for the presence of this activator in leukaemic cells according to the method of Cheung<sup>25</sup>. These cells did contain an activator of phosphodiesterase as evidenced by its ability to increase the activity of phosphodiesterase from mouse cerebrum fivefold (Table 2). It had no effect, however, on the phosphodiesterase from leukaemic lymphocytes. This suggested either that the phosphodiesterase from leukaemic lymphocytes could not be activated or that it was already maximally activated. To examine this latter possibility we measured the phosphodiesterase activity in the presence of the calcium chelator, EGTA. As can be seen (Table 2) EGTA produced a 75% inhibition of phosphodiesterase in brain (suggesting that this enzyme was already partially activated) but had no

**Table 2** Effects of phosphodiesterase activator from leukaemic lymphocytes on the cyclic AMP phosphodiesterase activity of cerebrum and leukaemic lymphocytes

Phosphodiesterase source	Cyclic AMP phosphodiesterase		
	Control	Activator	EGTA
Cerebrum	56 $\pm$ 4	217 $\pm$ 11*	16 $\pm$ 1*
Leukaemic lymphocytes	61 $\pm$ 3	59 $\pm$ 6	68 $\pm$ 3

Activator of phosphodiesterase was prepared from leukaemic lymphocytes according to Cheung<sup>25</sup>. Activity of phosphodiesterase was determined in whole sonicates of cerebrum of DBA/2 mice and L1210 leukaemic lymphocytes in the presence of 0.1 mM Ca<sup>2+</sup> and 3 mM Mg<sup>2+</sup> in the absence and presence of activator (25  $\mu\text{l}$ ) or EGTA (1 mM) using 250  $\mu\text{M}$  cyclic AMP as substrate. Activity is expressed as nmol cyclic AMP hydrolysed per mg protein per min. Each value represents the mean of four determinations  $\pm$  s.e.

\* $P < 0.001$  compared with control values.

effect on the phosphodiesterase of the leukaemic lymphocytes. In addition, when portions of enzyme preparations from normal lymphocytes were added to the enzyme from leukaemic cells, the phosphodiesterase activity was additive, suggesting that no inhibitor of phosphodiesterase was present in the normal cells. Thus, the greater activity of phosphodiesterase in leukaemic lymphocytes apparently was not caused by the presence of a phosphodiesterase more responsive to the endogenous activator or by a phosphodiesterase in a highly activated state, but rather was attributable to a greater quantity of the enzyme in the leukaemic cells.

Based on the present findings and on the evidence that cyclic AMP inhibits the rate of growth of these leukaemic lymphocytes<sup>26,27</sup>, one may speculate that the abnormal proliferation of these cells may be explained by an increase in the activity of the enzyme that catalyses the hydrolysis of cyclic AMP. The significance and interpretation of the high activity of cyclic GMP phosphodiesterase in leukaemic lymphocytes must await studies on the role of cyclic GMP in these cells. Further, since cyclic AMP phosphodiesterase exists in multiple forms<sup>21-23</sup>, which can be selectively inhibited by drugs<sup>28</sup>, it may be possible to inhibit specifically the predominant forms of the enzyme in these malignant cells. This might cause an elevation in the concentration of cyclic AMP, and consequently a reduction in the growth rate of the abnormal cells, leaving normal cells unaltered.

These studies on the multiple forms of phosphodiesterase as well as studies which are designed to show whether other qualitative differences exist between the phosphodiesterases of normal and leukaemic lymphocytes are currently in progress. It would be particularly instructive to determine, for example, if the increased activity is attributable to a single enzyme which hydrolyses both cyclic AMP and cyclic GMP or to a specific increase in an enzyme form with specificity for either of the two cyclic nucleotides.

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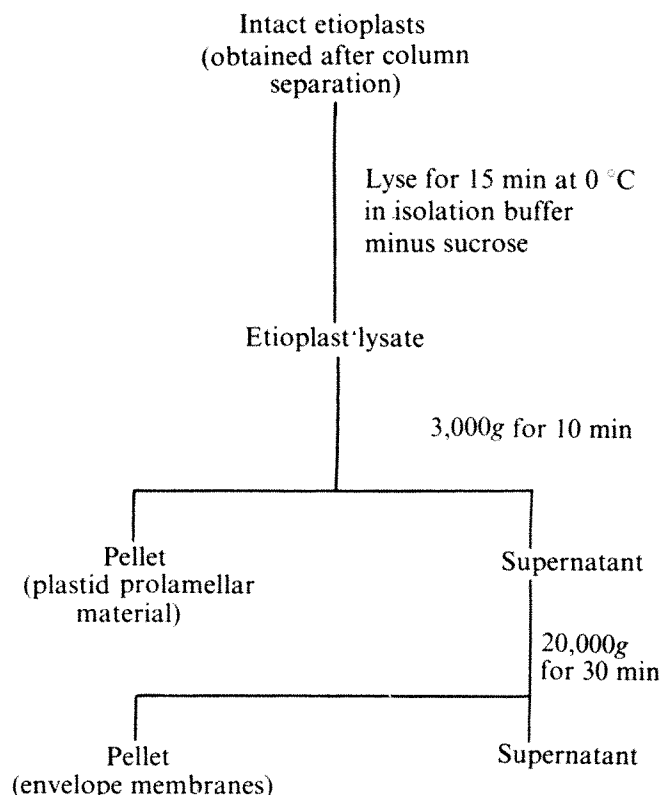
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## Spectrophotometric evidence for the presence of phytochrome in the envelope membranes of barley etioplasts

PHYTOCHROME is a chromoprotein photoreceptor, existing in two photoconvertible forms (Pr and Pfr), and is responsible for various metabolic and developmental responses of higher plants to light<sup>1</sup>. The location of phytochrome in plant cells and its mechanism of action are matters of debate. Many physiological responses to light have been adduced as evidence for phytochrome either being a component of certain cellular membranes, or acting on them, bringing about rapid changes in cell metabolism which lead to developmental changes<sup>2</sup>. There have been claims that phytochrome, in the Pfr form only, binds specifically to as yet uncharacterised membranous fractions<sup>3-6</sup> and the data have formed the basis for allosteric models of the action of phytochrome<sup>3-7</sup>. Further progress requires a demonstration of the precise location of phytochrome with relation to a well characterised membrane where it is known to direct a process *in vitro*. We demonstrated<sup>8,9</sup> the specific association of a proportion of total cellular phytochrome with etioplasts isolated from 6-d-old etiolated barley leaves. Etioplast phytochrome, as a result of its interconversions, was shown to regulate, *in vitro*, the efflux of endogenous etioplast gibberellins across the envelope membranes into the surrounding medium. Confirmatory results have been obtained with etiolated wheat leaves<sup>10,11</sup>. Although phytochrome was detected spectrophotometrically in the purified etioplast preparations from barley, no data were obtained

Fig. 1 Protocol for the separation of etioplast envelopes. Procedure as in ref. 10 modified by the use of 25 mM MOPS buffer (described in Table 1) as the isolation medium. The period of lysis at 0 °C was extended to 15 min.





**Table 1** Measurement of protein and phytochrome content at stages during isolation of etioplasts

Fraction	Protein per aliquot (mg)	$\Delta (\Delta A)$ 660/730 nm	$\Delta (\Delta A)$ per mg protein	Mean $\Delta (\Delta A)$ per mg protein	% Total phytochrome
(1) Crude homogenate	2.450	0.0100	0.00408	0.00447	100.00
	0.970	0.0040	0.00410		
	2.025	0.0100	0.00490		
	0.810	0.0038	0.00470		
	0.910	0.0042	0.00460		
(2) Crude plastid pellet	2.160	0.0028	0.00129	0.00140	1.48
	1.630	0.0022	0.00130		
	1.920	0.0025	0.00130		
	0.810	0.0015	0.00180		
	1.080	0.0014	0.00129		
(3) Supernatant	4.500	0.0205	0.00455	0.00450	89.45
	0.450	0.0020	0.00440		
	2.250	0.0105	0.00466		
	0.910	0.0040	0.00440		
(4) Washed plastid pellet	0.960	0.0024	0.0025	0.00210	1.32
	0.864	0.0018	0.0021		
	0.861	0.0022	0.0026		
	0.810	0.0015	0.0018		
	1.720	0.0030	0.0017		
(5) Supernatant	1.260	0.0000	0.0000	0.00000	0.00
	0.507	0.0000	0.0000		
	3.330	0.0000	0.0000		
	3.570	0.0000	0.0000		
	2.880	0.0000	0.0000		
(6) Purified etioplasts	0.720	0.0009	0.0013	0.00140	0.63
	0.390	0.0005	0.0013		
	1.590	0.0020	0.0013		
	1.800	0.0017	0.0009		
	1.320	0.0027	0.0021		

Etioplasts were isolated in 25 mM *N*-morpholino-3-propanesulphonic acid (MOPS) buffer containing 3 mM EDTA (disodium salt), 300 mM sucrose, 14 mM 2-mercaptoethanol and adjusted to a final pH of 7.5. Aliquots of (1) the crude homogenate, (2) the first crude plastid pellet, and (3) supernatant after a 1-min spin at 6,000g, (4) the washed plastid pellet and supernatant (5), after a second similar centrifugation, and (6) the final purified etioplasts, were taken for analysis. Samples of various volume were made up to 3 ml for dual wavelength measurement in a Perkin Elmer 156 digital double wavelength spectrophotometer. Measurement was made over a 1-cm path length using calcium carbonate as a light scattering agent. Actinic radiations lasting 30 s were of 660 nm and 730 nm. Measuring beams were also of these wavelengths.

for the suborganellar location of the photoreceptor or for the amount present in the etioplasts. We report here that although only a small proportion of the total phytochrome is associated with etioplasts, it seems to be concentrated in the envelope membranes. These data support the view that phytochrome is located in specific cellular membranes where it regulates transmembrane transport<sup>12</sup>.

Etioplasts were isolated and purified by a modification<sup>8,9</sup> of the Sephadex column technique developed by Wellburn<sup>13</sup>. As we have described previously<sup>8,9</sup>, this method yields etioplasts which are intact and very pure as judged by

electron microscopic and biochemical criteria. Samples for protein<sup>14</sup> and phytochrome<sup>8,9</sup> measurements were taken at each separation stage.

The data from five separate preparations are presented in Table 1, showing high reproducibility. The amount of phytochrome per mg protein of the purified etioplasts was between a quarter and a third that of the crude homogenate. According to Pratt and Coleman<sup>15</sup>, however, a large proportion of the phytochrome of etiolated tissues is in a free, soluble state in the cytoplasm and not associated with specific structures. Approximately 90% of the total homogenate phytochrome remained in the supernatant after the first centrifugation, although unknown amounts may have been associated with organelles or plastid fragments of a lower pelletability than intact etioplasts. Table 1 shows that the purified etioplasts accounted for about 2% of the homogenate protein and 0.6% of the total homogenate phytochrome. Although the actual proportion of phytochrome associated with the etioplasts is small, the number of possible phenomena which it may control there makes it far from insignificant<sup>1</sup>.

To determine the suborganellar location of etioplast phytochrome, purified etioplasts were fractionated into envelope, prolamellar and supernatant fractions according to the method of Mackender and Leech<sup>16</sup> modified as shown in Fig. 1. The prolamellar and envelope fractions contained the expected components revealed by electron microscopy, although biochemical characterisation has not been carried out. Measurements of protein and phytochrome in each fraction for two experiments are presented in Table 2. In both cases, although the protein contents of the 3,000g pellet (that is, the prolamellar fraction) and the 20,000g supernatant were roughly twice that of the 20,000g pellet

**Table 2** Measurement of phytochrome after the fractionation of etioplasts

Fraction	Protein (mg)	$\Delta (\Delta A)$ 660/730 nm	$\Delta (\Delta A)$ per mg protein	Mean $\Delta (\Delta A)$ per mg protein
Intact etioplasts	1.590	0.00200	0.00125	0.00167
	1.320	0.00270	0.00210	
3,000g pellet	1.115	0.00000	0.00000	0.00000
	1.115	0.00000	0.00000	
3,000g supernatant	1.080	0.00215	0.00199	0.00199
	1.000	0.00200	0.00200	
20,000g pellet	0.500	0.00120	0.00240	0.00241
	0.660	0.00160	0.00242	
20,000g supernatant	0.900	0.00000	0.00000	0.00000
	1.000	0.00000	0.00000	

Dual wavelength spectrophotometry was carried out on aliquots of samples made up to 3 ml.

(that is, the envelope fraction), phytochrome was only detectable in the latter. Approximately 77% of the initial etioplast phytochrome was recovered in the 20,000g pellet which in terms of protein content constitutes a 1.44-fold enrichment of phytochrome. The remaining phytochrome was lost or rendered undetectable.

Although definitive proof will depend on rigorous chemical and biochemical characterisation of the subetioplast fractions, these data strongly suggest that all etioplast phytochrome is located in the etioplast envelope. It seems unlikely that its presence there results from nonspecific adsorption, since our earlier work has shown that phytochrome exerts rapid control over transport of gibberellins across the etioplast envelope *in vitro*<sup>8,9</sup>. The pelletable phytochrome investigated by many other workers<sup>7</sup> seems to bind to membranous material only in the Pfr form; etioplast phytochrome, on the other hand, exists as Pr in or on the etioplast envelope. The characteristics of etioplast phytochrome, therefore, are not easily reconcilable with the concept of phytochrome existing as soluble Pr in the cytoplasm and moving to specific binding sites on membranes only when photoconverted to Pfr, as proposed in the allosteric models of phytochrome action<sup>7</sup>. The data are more consistent with our hypothesis<sup>8,9,12</sup> that phytochrome is a normal component of the membrane where it acts as a permease, or transport factor, to regulate the movement of one or more critical metabolites between compartments.

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## Regulation of RuDP carboxylase/oxygenase activity and its relationship to plant photorespiration

RIBULOSE 1,5-diphosphate carboxylase/oxygenase (fraction 1 protein), the most abundant protein in nature, is a key photosynthetic enzyme catalysing the addition of CO<sub>2</sub> to ribulose 1,5-diphosphate (RuDP) to form two molecules of phosphoglyceric acid (PGA). The enzyme also has a competitive oxygenase function catalysing the splitting of RuDP by O<sub>2</sub> to form one molecule of PGA and one molecule of phosphoglycolate<sup>1–3</sup>. The latter function of this enzyme has been suggested to be the primary source of phosphoglycolate, the principal substrate for photorespiration<sup>5</sup>.

Fraction 1 protein consists of eight large and eight small subunits<sup>6</sup>. The large subunits are both encoded and synthesised within the chloroplast<sup>7,8</sup> and seem to function as

the catalytic sites for the carboxylase/oxygenase functions of this enzyme<sup>9</sup>. In contrast, the small subunits are both encoded and synthesised outside the chloroplast and their function is unresolved<sup>10</sup>. In this report, we present evidence that the small subunits regulate the carboxylase/oxygenase activity of fraction 1 protein<sup>9</sup> and that the oxygenase function of this enzyme is probably not the principal source of phosphoglycolate *in vivo*<sup>5</sup>.

We prepared crystallised fraction 1 protein<sup>11</sup> from green, wild-type (*su/su*) tobacco leaves (cv John Williams Broadleaf) and from the heterozygous, dominant, yellow mutant (*Su/su*) of this line. Mature leaves were obtained from green and yellow progeny derived by selfing the heterozygous yellow plant. Two important features of the yellow plants are that they result from a nuclear mutation<sup>12</sup> and that yellow plants have a two to threefold higher rate of photorespiration than the wild-type sibling<sup>13,14</sup>.

The polypeptide patterns of the fraction 1 protein isolated from the wild type and yellow mutant sibling leaves are shown in Fig. 1. Both proteins had identical isoelectric focusing patterns consisting of three large and two small subunit polypeptides. Thus, we found no detectable (by this technique) structural change of the small subunit between the wild and mutant proteins. As Table 1 shows, however, RuDP carboxylase/oxygenase activities are markedly different. Carboxylase/oxygenase activities of the fraction 1 protein from the mutant were about 50% lower than those obtained with the wild-type enzyme. It should be noted that intermediate levels of activity were observed with a mixture (1:1) of the two enzymes.

A lack of any detectable difference in the small subunit structure between the wild type and its mutant was not entirely unexpected. First, no intraspecific variations in fraction 1 protein have been observed on isoelectric focusing gels in 8 M urea<sup>7</sup>. Second, changes in amino acid composition or sequence without a change in overall net charge of the polypeptides would not be detected by this method.

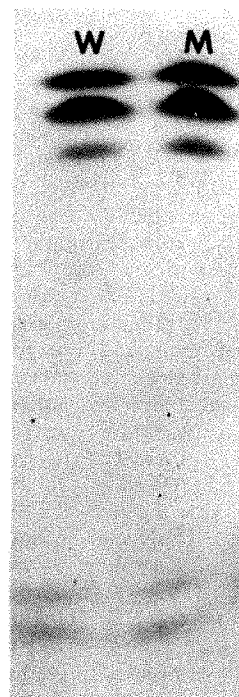


Fig. 1 Polypeptide composition of fraction 1 protein from wild type (W) and yellow mutant (M) of John Williams Broadleaf, a cultivar of *N. tabacum*. The crystalline fraction 1 proteins were s-carboxymethylated before applying to a prefocused 4.5% polyacrylamide slab gel containing 1% ampholine (pH 5–7) and 8 M urea. Isoelectric focusing was performed as described previously<sup>15</sup>.

**Table 1** RuDP-carboxylase/oxygenase activities of crystallised fraction 1 protein from John Williams Broadleaf wild type (*su/su*) and yellow mutant (*Su/su*) leaves

Experiment	Fraction 1 protein	Carboxylase activity* (c.p.m. $\times 10^2$ per mg protein)	Relative carboxylase activity (%)	Oxygenase activity† (nmol O <sub>2</sub> per mg protein per min)	Relative oxygenase activity (%)
1	Wild type ( <i>su/su</i> )	414.3	100	25.8	100
	Mutant ( <i>Su/su</i> )	236.5	57	10.1	39
	Mixture ( <i>su/su</i> : <i>Su/su</i> ) (1:1 mixture)	317.2	76	17.0	66
	Wild type ( <i>su/su</i> )	425.0	100	28.3	100
2	Mutant ( <i>Su/su</i> )	242.0	56	11.4	40

Experiments 1 and 2 were done with crystalline fraction 1 protein obtained from separate progeny.

\*RuDP carboxylase activity was determined by the  $\text{H}^{14}\text{CO}_3^-$  fixation assay<sup>16</sup>.

†RuDP oxygenase activity was measured polarographically<sup>17</sup>.

Recent results indicate that serological methods can distinguish differences in fraction 1 protein polypeptide composition not resolved by the isoelectric focusing technique (J. C. Gray, personal communication).

The difference in carboxylase and oxygenase activity observed for crystallised fraction 1 protein from the wild type and yellow mutant variety of tobacco was highly reproducible (Table 1). It should be noted that the yellow plant is a nuclear mutant and that it is the small subunit of fraction 1 protein which is nuclear encoded<sup>7</sup>. The difference in activities cannot be attributed to changes in the large subunits (which are maternally encoded) since the proteins were prepared from progeny derived from one parent. Any maternal influence should be expressed equally in all siblings. Thus, our results suggest that the small subunit modifies or regulates enzymatic activity of this protein. How this is accomplished remains to be established.

A surprising outcome of these experiments was the apparent lack of complete correlation between the specific carboxylase/oxygenase activities of the isolated fraction 1 proteins and the respective physiological responses of the plant. For example, the yellow mutant, grown under moderate intensities of light and normal atmospheric levels of  $\text{CO}_2$ , characteristically has a higher photorespiratory rate (~threefold) and a lower net rate of photosynthesis, compared with the wild type<sup>13,14</sup>. We note from the data in Table 1 that (1) the carboxylase and oxygenase activities of fraction 1 protein seem to be tightly coupled functions; (2) the carboxylase activities of the isolated enzymes and the respective photosynthetic yields of the parent plants are correlated, and (3) the oxygenase activities of the isolated fraction 1 proteins and the respective photorespiratory rates of the parent plants are not correlated. Based on these data, we suggest that the small subunit regulates the oxygenase and carboxylase function of fraction 1 protein concomitantly rather than independently. Lorimer and Andrews<sup>18</sup> earlier proposed on theoretical grounds that the two functions of this enzyme could not be separated. Our results also support the proposal that RuDP-oxygenase is not the sole source of phosphoglycolate<sup>3</sup>.

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## Increase in serum haptoglobin stimulated by prostaglandins

SERUM haptoglobin is decreased in haemolytic anaemias and severe hepatic dysfunction such as cirrhosis, and increased in many pathological states, especially inflammatory and malignant neoplastic disorders<sup>1</sup>. Serum haptoglobin can be increased markedly in experimental animals by various inflammatory agents such as turpentine, carrageenan or proteolytic enzymes<sup>2</sup>. Others, such as histamine and 5-hydroxytryptamine, produce a very slight increase<sup>3</sup>. I have now found that various prostaglandins ( $\text{PGE}_1$ ,  $\text{PGE}_2$ ,  $\text{PGF}_{2\alpha}$  and  $\text{PGA}_2$ ) can increase serum haptoglobin as much as do turpentine and carrageenan.

Willis<sup>4</sup> showed that PGEs are increased in inflammatory exudate, and Hammerström *et al.*<sup>5</sup> found increased  $\text{PGE}_2$  in fibroblasts transformed by polyoma virus. In my experiments  $\text{PGE}_1$  (as little as  $1 \mu\text{g kg}^{-1}$ ) produced more than a fivefold increase of serum haptoglobin in rabbits;  $2 \mu\text{g kg}^{-1}$  produced more than a tenfold increase 24 h after injection (Fig. 1a). A larger dose produced a more marked response. Repeated injection produced a prolonged increase, as in pathological states (Fig. 1b). This response differs from that observed in response to turpentine, which had the same effect when injected repeatedly as when injected once<sup>2</sup>.

The effect of PG was inhibited by previous administration of cycloheximide, 5-fluorouracil or ethionine (Fig. 1c), which suggests that PG stimulates the synthesis of haptoglobin in the liver. On the other hand, the action of turpentine was not inhibited by these agents (Fig. 1c), suggesting that turpentine releases stored haptoglobin in the liver. Only previous treatment with carbon tetrachloride inhibited the action of turpentine<sup>6,7</sup>. Carbon tetrachloride seems to block the passage of haptoglobin from the hepatic pool into the circulation<sup>8</sup>.  $\text{PGE}_1$  increases the

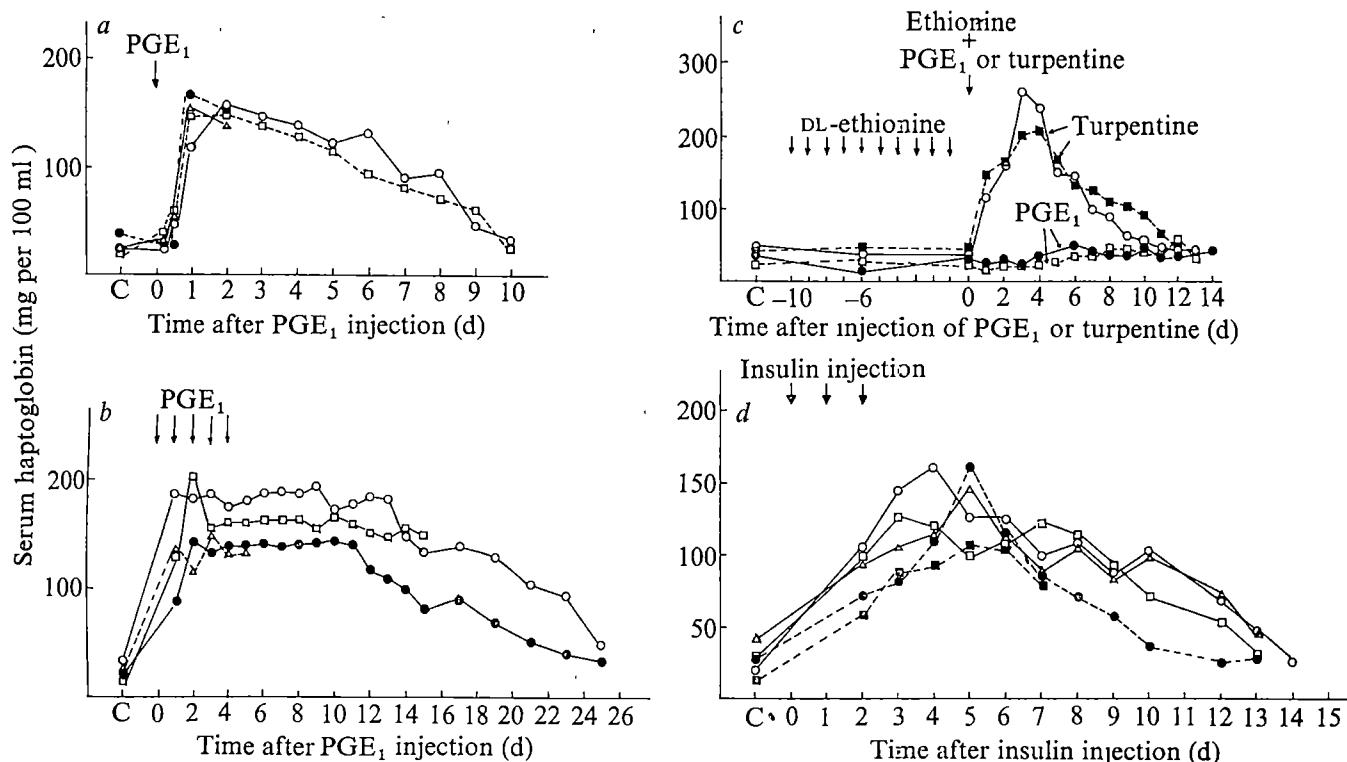


Fig. 1 *a*, Effect of intravenous PGE<sub>1</sub> (2 μg kg<sup>-1</sup>) on serum haptoglobin level in four rabbits. PGE<sub>1</sub> (5 mg) was dissolved in 0.5 ml of 95% ethanol, and mixed with 4.5 ml of 0.02% Na<sub>2</sub>CO<sub>3</sub> solution. The stock PGE<sub>1</sub> solution (1 mg ml<sup>-1</sup>) was divided into samples of 0.05 ml (50 μg of PGE<sub>1</sub>) to vials, and kept frozen until use. The stock PGE<sub>1</sub> solution was diluted with 0.15 M NaCl immediately before use. Serum haptoglobin was measured by paper electrophoresis<sup>13</sup>. Maximum haptoglobin was reached after 24 h and returned to control level after 10 d. The pattern is very similar to that observed after injection of turpentine. PGE<sub>1</sub> (30 μg kg<sup>-1</sup>) increased rabbit serum haptoglobin as much as 300 mg per 100 ml of serum. When PGE<sub>1</sub> was administered subcutaneously, the effect was much less prominent. A similar effect was obtained with PGE<sub>2</sub>, PGF<sub>2α</sub> or PGA<sub>2</sub> but with less marked effect. *b*, Effect of prolonged administration of PGE<sub>1</sub> (2 μg kg<sup>-1</sup> for 5 d) on serum haptoglobin in four rabbits. *c*, Effect of DL-ethionine (10 mg kg<sup>-1</sup>) on PGE<sub>1</sub> and action of turpentine on serum haptoglobin. Ethionine was administered intraperitoneally to four rabbits for 10 d. On the eleventh day PGE<sub>1</sub> (30 μg kg<sup>-1</sup>) was injected with ethionine into two rabbits. The remaining two rabbits were injected with 1 ml of turpentine subcutaneously at the dorsolumbar region. Cycloheximide (2 mg kg<sup>-1</sup>) was injected for 5 d and 5-fluorouracil (10 mg kg<sup>-1</sup>) was injected intraperitoneally. *d*, Effect of insulin (0.5 U kg<sup>-1</sup> d<sup>-1</sup>), administered intramuscularly for 3 d.

content of cyclic AMP in the liver, whereas PGF<sub>2α</sub> and PGA<sub>1</sub> have no effect<sup>9,10</sup>. Insulin, which causes a decrease of cyclic AMP in the liver<sup>11</sup>, also increased serum haptoglobin quite strikingly in my experiments (Fig. 1*d*). Thus PG may stimulate haptoglobin synthesis directly in the liver, or act in some other way not involving cyclic AMP.

Whereas the biological actions of PGs differ according to the type of PG and tissue involved<sup>12</sup>, the fact that serum haptoglobin was increased by every PG tested, irrespective of structure, is interesting. In any case, serum haptoglobin level provides a convenient marker for various actions of PG *in vivo* as long as the function of the liver is intact. The biological significance of increased haptoglobin in the serum remains to be elucidated.

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## Exclusively inhibitory action of iontophoretic acetylcholine on single neurones of feline thalamus

In most regions of the mammalian brain only a small percentage of the neurones located with multi-barrelled micropipettes are inhibited by iontophoretic acetylcholine (ACh). Indeed, the much more frequent occurrence of cells excited by rather small doses of acetylcholine (see ref. 7 for review), together with the finding that both the inhibitory and excitatory actions of ACh are often antagonised by the same pharmacological agents, has led to the suggestion that the inhibitory action of ACh is mediated indirectly through the excitation of neighbouring cholinceptive interneurons whose inhibitory transmitter is a substance other than ACh<sup>2,3</sup>. Several workers<sup>4-8</sup> have tried to overcome this difficulty by working in the most superficial layers of the cerebral cortex where a strong inhibitory action of ACh predominates and ACh excitation has seldom been reported. We wish to draw attention to the possibility that the nucleus reticularis of the cat thalamus may well prove to be an appropriate area in which to study the depressant action of ACh on central mammalian neurones since almost every cell encountered proved to be inhibited by a short, low amplitude pulse of ACh.



Four cats anaesthetised with a mixture of halothane (0.5%), nitrous oxide (65%) and oxygen were placed in a stereotaxic frame and burr holes prepared in both sides of the skull at the appropriate stereotaxic coordinates. Through each burr hole a single track was made using a freshly filled five barrelled micropipette which contained one barrel filled with 4 M NaCl for single unit recording, another with 1 M NaCl for current and current balancing controls and two drug barrels containing 1M AChCl and 1 M sodium glutamate. In this preliminary study no other drugs or putative inhibitory transmitters were present in the microelectrodes. The coordinates of the electrodes were AP9.5 and L7.0 or 8.0 and recording began at H7.0 and continued until H0.0 (ref. 9). After a single microelectrode track had been made on each side of the brain the animals were more deeply anaesthetised with pentobarbitone, killed by perfusion fixation, and the brains prepared for histological analysis.

In Fig. 1*b* five microelectrode tracks, made in four cats on the stereotaxic coordinates AP9.5 and L7.0 or 8.0, are represented schematically by vertical lines to show the depth of the neurones which were either inhibited (●) or excited (×) by small iontophoretic applications of ACh as the microelectrodes transversed first the nucleus reticularis and then entered the ventrobasal complex of the thalamus. Track 1, which lay within the thickness of a single histological section and from which the line drawn in Fig. 1*a* was traced, penetrated only the nucleus reticularis and located only cells inhibited by ACh. All other tracks lay slightly more medial to track 1 and penetrated both the nucleus reticularis and the ventral basal complex of the thalamus. Tracks 4 and 5 are from the same cat. In track 4 the results suggest that the electrode re-entered the nucleus reticularis at the lower limit of its descent and again located cells inhibited by ACh. During the withdrawal of this electrode through the nucleus reticularis three more

cells inhibited by ACh (○) were found. During a subsequent descent of the same electrode on the right side of the cat (track 5), the depth distribution of cells inhibited and excited by ACh was almost identical to that seen in track 4. This experiment eliminates the possibility that the change from ACh-evoked inhibition to excitation reflects a progressive deterioration in either the health of the animal, or the effectiveness of the microelectrode and its contents. The oscillographs in Fig. 2 illustrate the changes in single unit activity of two neurones located in track 4 during iontophoretic applications of ACh. The first cell located in the nucleus reticularis at the approximate stereotaxic coordinate H4.41 shows the typical spontaneous activity of all cells located in this region and was strongly inhibited by ACh. The second neurone which lay approximately 2.43 mm deeper than the nucleus reticularis shows the typical excitation evoked by iontophoretic ACh applied to a majority of neurones in the ventrobasal thalamus<sup>10</sup>. The ACh-evoked inhibition could not be mimicked by the passage of larger amounts of positive current through the NaCl barrel of the microelectrode and was only marginally reduced in intensity when the current through the ACh barrel was balanced by passing a negative current of equal magnitude through the NaCl barrel. Of 74 cells found to be inhibited in the five tracks shown schematically in Fig. 1*a* (cells less than 50  $\mu$ m apart and showing the same response to ACh are represented by a single symbol) 17 were inhibited when the retaining current on the ACh barrel (25 nA) was reduced and 57 more by currents of less than 40 nA ( $33 \pm 3$  nA).

In addition, the inhibition evoked by the release of ACh by relatively low current applications had a shorter latency of onset than the excitations evoked by either the same or larger currents from the same microelectrode during its descent through the deeper nuclei of the thalamus. The observations that the onset of ACh-evoked inhibition is of

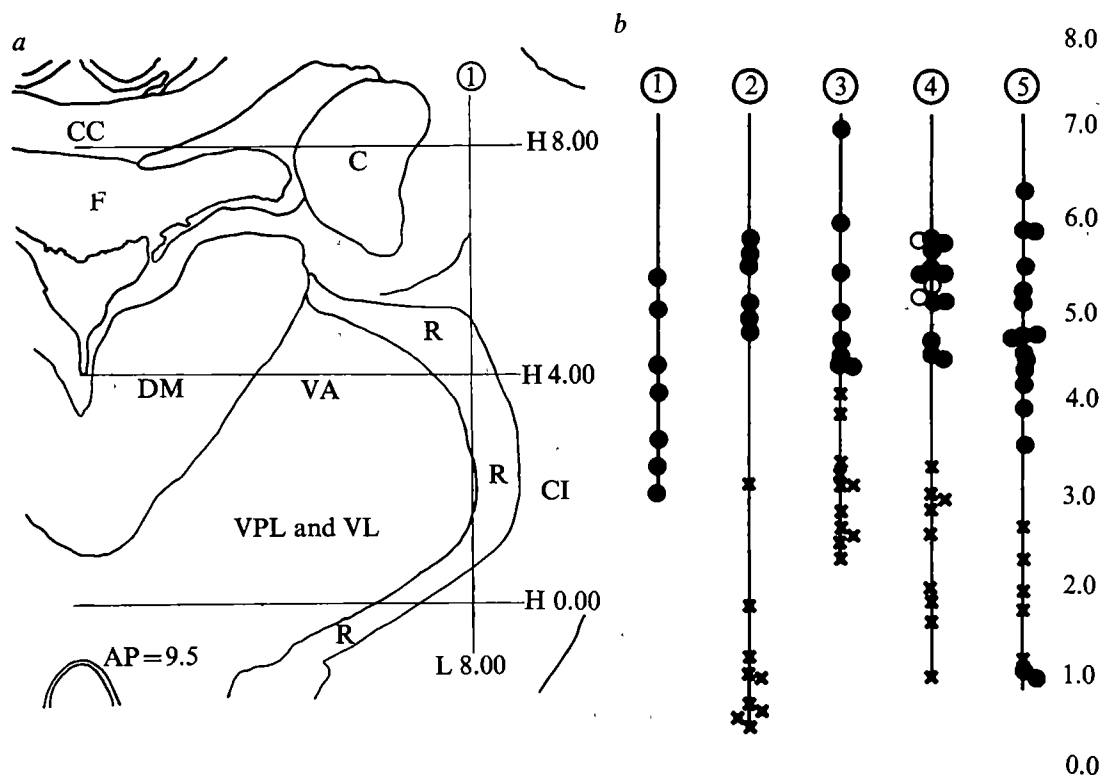
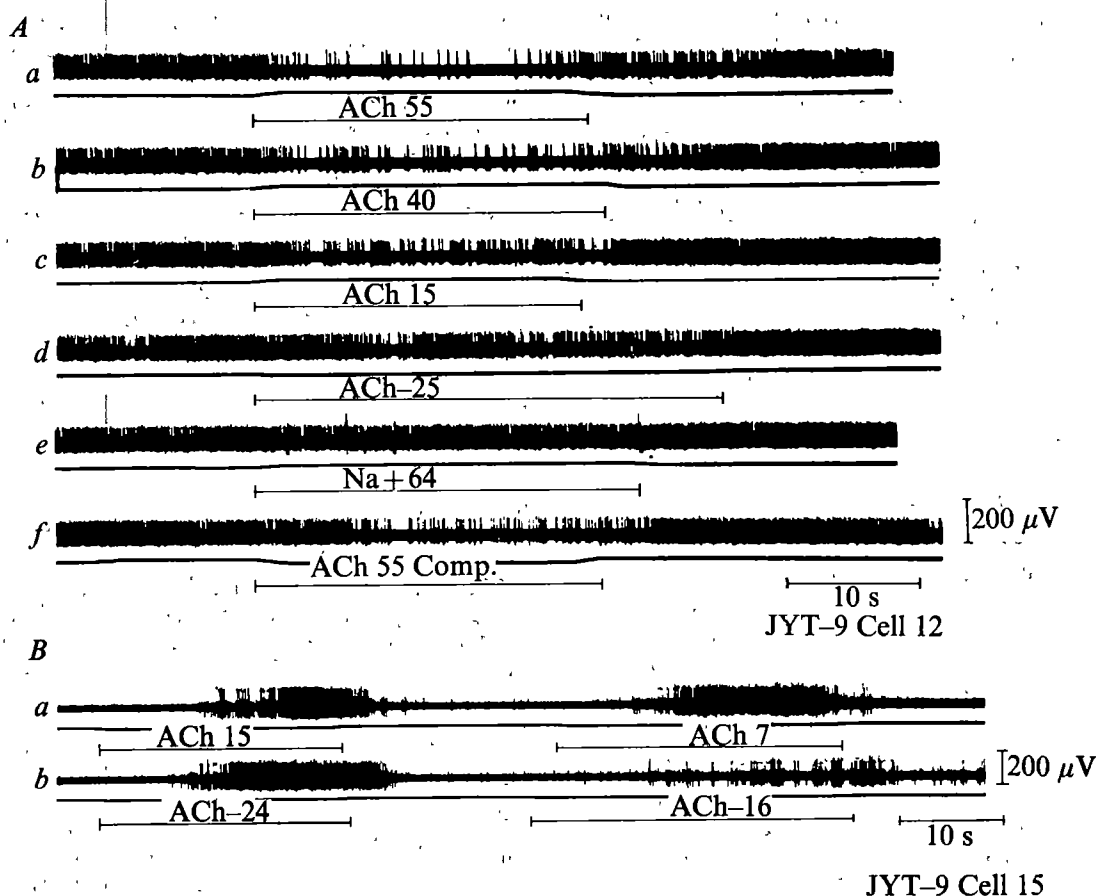


Fig. 1 Schematic drawings to show how cells located within the nucleus reticularis were inhibited by iontophoretic ACh and those lying deeper in the ventrobasal complex of the thalamus excited. *a*, From the histological section which contained the microelectrode track represented by line 1 in *b* shows line L8.00 down which this particular microelectrode passed. The five vertical lines in *b* schematically show the depth distribution of the cells which were either inhibited (●) or excited (×) as microelectrodes penetrated or were withdrawn (○) from the brain of four cats along the coordinates AP9.5 and L7.0 (2 and 3) or 8.0 (1, 4 and 5). Tracks 4 and 5 are from the left and right side of the same cat. The depths of the cells along each track were read from the micrometer of the micro-manipulator drive and no attempt has been made to correct these values so that they correspond with the coordinates taken from the atlas of Snider and Niemer<sup>9</sup> and arbitrarily assigned to the line drawing.



**Fig. 2** Oscillographic records to show the inhibition and excitation of neurones in the nucleus reticularis and ventrobasal complex of the thalamus located in the same microelectrode track. *A*, From a cell located at the stereotaxic coordinate H4.41 showing: *a-c*, dose-related inhibitions evoked by progressively smaller doses of ACh released by currents from 55–15 nA; *d*, effect of withdrawing 25 nA of the 25 nA backing current; *e*, lack of effect of 60 nA positive current passed through NaCl barrel; *f*, effect of balancing the 55 nA passed through the ACh barrel by –55 nA passed through the NaCl barrel. *B*, Records *a* and *b* show excitation of a neurone located 2.43 mm deeper in the track by similar doses of ACh. Note that the delay between the onset of the ACh application and the ACh-evoked excitation in *B* was greater than that which occurred in *A* between the onset of the ACh pulse and the ACh-evoked inhibition.

shorter latency than ACh-evoked excitation are in keeping with the view of Krnjević<sup>1</sup>, who has suggested that ACh-evoked excitation in the cerebral cortex is often preceded by a period of inhibition. Since in the nucleus reticularis ACh-evoked inhibition has a shorter latency than the excitation evoked by larger currents in the ventrobasal complex, it is difficult to believe that ACh-evoked inhibition could be mediated by the excitation of an intervening cholinergic interneurone. The possibility that the inhibitory action of ACh might be mediated by a presynaptic mechanism was eliminated in part by showing ACh to be an equally effective inhibitor of cells when firing was enhanced by glutamic acid.

The finding that the action of ACh in the thalamus is excitatory in one region and inhibitory in another could be of great functional significance. For instance it now seems possible that a single cholinergic pathway could excite neurones of the ventrobasal complex and inhibit neurones of the nucleus reticularis. This 'push-pull' arrangement is in keeping with the neurophysiological data of Schlag and Wazak<sup>21</sup> and Massion and Rispal-Padel<sup>22</sup>, which has led to the suggestion that the firing of the neurones of the ventrobasal complex is modulated by inhibitory interneurones whose cell bodies lie within the nucleus reticularis. This idea is contrary, however, to Jasper's<sup>13</sup> original hypothesis which supposed that the nucleus reticularis was the 'final relay' nucleus of the ascending excitatory pathway involved in 'cortical arousal'. Moreover, modern anatomical studies of the thalamus have not been able to confirm the presence of a pathway from the nucleus reticularis to the cortex and indeed show the

majority of the neurones of this nucleus to project to the ventrobasal complex of the thalamus<sup>14–16</sup>. Thus Jasper's original view<sup>13</sup> that the nucleus reticularis is intimately involved in cortical arousal can only be retained by assuming that the neurones of the nucleus reticularis are inhibited by the ascending cholinergic pathway and thus bring about cortical arousal indirectly through disinhibition of the neurones of the more medial nuclei of the thalamus. Interestingly enough, similar physiological findings<sup>17,18</sup> have led Krnjević<sup>1</sup> to suggest that the ACh-inhibited cells in the upper layers of the cerebral cortex bring about disinhibition of ACh-excited neurones lying much deeper in the cerebral cortex. An analogous situation exists in the nervous system of *Aplysia*, in which the firing of a single cholinergic neurone has been shown to excite one cell and inhibit another<sup>19,20</sup>.

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## Formation of oestradiol-17 $\beta$ from oestrone sulphate by sheep foetal pituitary *in vitro*

OESTROGENS are synthesised in large amounts by the placenta in several species including man and domestic animals. They occur in the foetal circulation as inactive sulphoconjugates<sup>1</sup> formed by a detoxifying mechanism that protects the foetus from the detrimental effects of high levels of these potent hormones. In the adult sheep, oestradiol-17 $\beta$  exerts feedback effects on gonadotrophin secretion by the adenohypophysis, as in other mammals<sup>2</sup>. In the foetal lamb the pituitary and adrenal are essential in the onset of parturition for hypophysectomy results in the prolongation of gestation, and the infusion of adrenocorticotrophic hormone (ACTH) or glucocorticoids induce delivery<sup>3</sup>. The factors that regulate the activity of the foetal adenohypophysis in late pregnancy are unknown.

A study of the steroid metabolising capacity of the foetal and maternal adenohypophysis and hypothalamus has revealed that oestrone sulphate, present in foetal circulation, can act as a prohormone since it is readily converted *in vitro* into the more biologically active oestrogens, oestrone and oestradiol-17 $\beta$ , especially in the foetal adenohypophysis.

Ewes were anaesthetised with fluothane and foetuses were removed by Caesarean section. After exsanguination under anaesthesia the maternal and foetal adenohypophysis, hypothalamus and a portion of the frontal cortex were removed and weighed. Tissue was minced and incubated in 2 ml of buffer (0.02 M Tris-HCl, 0.32 M sucrose, 0.001 M

MgCl<sub>2</sub> and 0.005 M NADPH, pH 7.4) at 37 °C for 2 h in 95% oxygen, 5% carbon dioxide. Incubations contained 1  $\mu$ Ci of either 6,7-<sup>3</sup>H-oestrone sulphate (2.13 nmol, 470 mCi mmol<sup>-1</sup>) or 6,7-<sup>3</sup>H-oestrone (0.022 nmol, 45 Ci mmol<sup>-1</sup>). After incubation labelled oestrogens were isolated by phenolic extraction, solvent partition and thin-layer chromatography and their radiochemical purity was established by recrystallisation to constant specific activity.

<sup>3</sup>H-oestrone sulphate was hydrolysed to oestrone and converted to oestradiol-17 $\beta$  in all tissues studied (Tables 1 and 2). The arylsulphatase enzymatic activity was about three times greater in the adenohypophysis of the foetus than in that of the ewe ( $P < 0.02$ ). The activity in the hypothalamus and the frontal cortex of both foetus and ewe was low, being similar to that observed in the maternal adenohypophysis. In all tissues, labelled oestrone and oestradiol-17 $\beta$  formed from oestrone sulphate accounted for the total radioactivity recovered in the phenolic fraction. There was no evidence of sulphotransferase activity in any of the tissues studied when <sup>3</sup>H-oestrone was used as substrate.

The quantity of unconjugated oestrogens produced from <sup>3</sup>H-oestrone sulphate by the adenohypophysis *in vitro* was substantial. This was exemplified by an experiment at parturition in which foetal pituitary tissue incubated with <sup>3</sup>H-oestrone sulphate (423 ng per ml medium) yielded the equivalent of 284 ng oestrone and 12.6 ng oestradiol-17 $\beta$  from one pituitary during 2 h of incubation. There was no change in the level of arylsulphatase activity of the foetal adenohypophysis during the second half of gestation or at the time of the sharp increase in plasma conjugated oestrone sulphate that occurred shortly before parturition<sup>4</sup>.

The formation of oestradiol-17 $\beta$  from <sup>3</sup>H-oestrone sulphate demonstrated the presence of 17 $\beta$ -oxidoreductase activity. When foetal and maternal tissues were incubated with <sup>3</sup>H-oestrone instead of <sup>3</sup>H-oestrone sulphate the activity of the foetal adenohypophysis was found to be approximately seven times that of the maternal adenohypophysis ( $P < 0.005$ , Fig. 1). In the hypothalamus and frontal cortex of the foetal lamb and ewe, 17 $\beta$ -oxidoreductase activity was low and similar to that in the maternal pituitary.

The enzymatic capacity of the maternal hypothalamus and adenohypophysis to hydrolyse and convert oestrone sulphate to oestrone and oestradiol-17 $\beta$  confirms results in anaestrous sheep, and has been implicated in the mechanisms by which pituitary function is regulated<sup>5</sup>. Arylsulphatase and 17 $\beta$ -oxidoreductase activities in the central nervous system and adenohypophysis of the foetal lamb have not been previously reported. These enzymes may lead

**Table 1** <sup>3</sup>H-oestrone sulphate metabolised to oestrone and oestradiol-17 $\beta$  by foetal and maternal tissues from the adenohypophysis and central nervous system

Tissue	Source	Days pregnant	
		81-126	131-145
Oestrone sulphate-oestrone			
	Adenohypophysis		
	Foetal	1,121.5 $\pm$ 218.5(3)	1,195.2 $\pm$ 386.6(3)
	Maternal	326.0 $\pm$ 120.4(3)	856.9 $\pm$ 147.6(4)
Hypothalamus	Foetal	703.4 $\pm$ 90.2(3)	794.7 $\pm$ 121.5(3)
	Maternal	653.9 $\pm$ 154.9(3)	856.9 $\pm$ 147.6(4)
Frontal cortex	Foetal	461.8 $\pm$ 121.9(3)	887.8 $\pm$ 62.8(3)
	Maternal	601.7 $\pm$ 51.5(2)	623.5 $\pm$ 178.6(4)
Control, no tissue		< 3.0	< 3.0
Oestrone sulphate-oestradiol-17 $\beta$			
	Adenohypophysis		
	Foetal	129.1 $\pm$ 22.2(3)	146.9 $\pm$ 50.0(3)
	Maternal	54.8 $\pm$ 22.4(3)	65.4 $\pm$ 45.2(4)
Hypothalamus	Foetal	34.0 $\pm$ 6.4(3)	35.2 $\pm$ 4.2(3)
	Maternal	50.6 $\pm$ 14.5(3)	59.8 $\pm$ 23.3(4)
Frontal cortex	Foetal	20.6 $\pm$ 6.5(3)	26.0 $\pm$ 5.1(3)
	Maternal	27.5 $\pm$ 13.0(3)	25.2 $\pm$ 9.5(4)
Control, no tissue		< 0.6	< 0.6

Figures are pmol per 100 mg tissue per 2 h and represent mean  $\pm$  s.e.m. No. of animals are given in parentheses.

**Table 2**  $^3\text{H}$ -oestrone and  $^3\text{H}$ -oestradiol-17 $\beta$  formed from  $^3\text{H}$ -oestrone sulphate; specific activity during successive crystallisations in acetone-*n*-pentane

			$^3\text{H}$ -oestrone (d.p.m. mg $^{-1}$ )			$^3\text{H}$ -oestradiol-17 $\beta$ (d.p.m. mg $^{-1}$ )				
			1†	2	3	1	2	3	4	5
Adenohypophysis	Foetal*	C	34,379	37,890	37,763	712	440	665	575	725
		ML	45,823	31,132	34,031	576	541	830	548	513
	Maternal	C	59,186	62,465	68,592	4,603	4,887	4,828	4,863	5,293
		ML	78,580	52,854	71,290	4,645	5,012	6,576	4,064	4,204
Hypothalamus	Foetal	C	58,130	68,073	71,844	753	1,081	1,026	1,014	—
		ML	74,564	74,760	66,837	959	1,438	1,000	892	555
	Maternal	C	175,514	163,868	170,409	8,636	8,682	7,916	8,964	8,262
		ML	172,540	192,987	165,821	7,230	7,823	10,880	10,305	12,339
Frontal cortex	Foetal	C	11,236	9,990	13,336	1,217	629	546	469	—
		ML	18,783	10,375	13,098	331	1,112	563	409	533
	Maternal	C	158,664	152,782	170,158	3,883	—	—	—	—
		ML	233,111	163,732	156,596	2,181	—	—	—	—

Oestrogens were isolated from tissue incubations by phenolic extraction, solvent partition and thin-layer chromatography before crystallisation.

\*Upper-figure crystals (C), lower figure mother liquor (ML).

† Crystallisation number.

—, Not determined.

to the local formation of appreciable amounts of oestrogens from conjugated compounds, such as oestrone sulphate, known to be present in the foetal circulation at a concentration of 2–4 ng ml $^{-1}$  in late pregnancy. Shortly before delivery the plasma concentration of oestrone sulphate in

the foetus increases to even higher levels (up to 39 ng ml $^{-1}$  (ref. 4)), and this change, together with the capacity of the foetal adenohypophysis to produce oestradiol-17 $\beta$ , may contribute to the cascade of endocrine events associated with the onset of parturition.

Other workers have postulated that oestrogens formed by aromatisation in the central nervous system and adenohypophysis of the adult rabbit and developing human foetus may be concerned in sexual differentiation, indicated by the higher conversion of androstenedione to oestrone in the male than in the female<sup>6</sup>. In the sheep we have found no evidence of aromatisation in similar tissues during the second half of pregnancy, and there was no significant sex difference in the arylsulphatase or 17 $\beta$ -oxidoreductase activities. It should be noted, however, that sexual differentiation in the sheep occurs during the first third of the 145-d gestation period<sup>7</sup>. The local formation of oestrogens from oestrogen sulphoconjugated compounds seems to comprise an alternative pathway for the production of these potential regulatory hormones within the central nervous system and adenohypophysis.

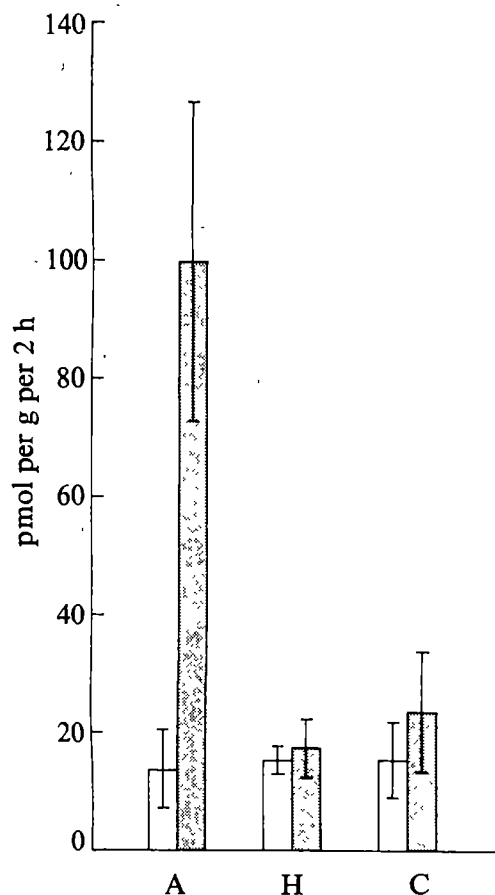
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**Fig. 1** Formation of oestradiol-17 $\beta$  from oestrone by the adenohypophysis (A), hypothalamus (H) and frontal cortex (C) of foetal lambs (shaded columns) and adult sheep (open columns) during the last third of pregnancy. The mean  $\pm$  s.e.m. of the conversion in four animals is expressed as pmol oestrone converted per 100 mg tissue during a 2 h incubation.

## Enzyme induction in mammalian cells defective in 28S ribosomal RNA formation

SYNTHESIS of ribosomal RNA and its transfer to the cytoplasm have been implicated repeatedly in the processing and transport



of informational RNA and the regulation of enzyme activities<sup>1-4</sup>. We have studied the involvement of rRNA in the RNA synthesis-dependent induction of the microsomal enzyme, aryl hydrocarbon hydroxylase<sup>5</sup>; the activity of this mixed-function oxygenase in mammalian tissues and in cells in culture increases many-fold after a few hours exposure to a polycyclic hydrocarbon<sup>6-10</sup>. The induction process requires *de novo* protein synthesis and DNA-dependent RNA synthesis<sup>5,9,11</sup> and can be influenced by post-transcriptional regulatory mechanisms<sup>9,12</sup>.

Our observations suggested that the induction-specific RNA is a heterogeneous, informational RNA which is transferred to and functions in the cytoplasm independently of concomitant rRNA synthesis and transfer<sup>5</sup>. These observations were based on the use of the inhibitors actinomycin D and cycloheximide, both of which may interfere with several aspects of the regulation of aryl hydrocarbon hydroxylase activity<sup>5,9,11,12</sup>. To avoid the ambiguities associated with the use of the inhibitors, we have now examined the possible role of newly formed rRNA in the induction process using a mutant cell line of BHK cells which is temperature-sensitive for the production of 28S rRNA<sup>13</sup>.

Mutant BHK-422E cells were obtained from Dr Claudio Basilico and cultured in medium composed of Eagles' minimal essential medium, 5% foetal bovine serum, 10% tryptose broth (Difco), penicillin, streptomycin, tylosin and gentamycin. They were grown at either 34 or 39 °C for 24 h before rRNA synthesis was examined. <sup>3</sup>H-uridine was added during a subsequent 2-h incubation and cytoplasmic RNA was extracted and analysed by electrophoresis in polyacrylamide-Agarose mixed gels. In cells grown at the permissive temperature (34 °C), two electrophoretic peaks appeared which corresponded to the 18S and 28S ribosomal RNA (Fig. 1). The amount of newly synthesised 28S RNA was about equal to that of 18S rRNA in cells grown at 34 °C. With longer labelling periods, the radioactivity in the 28S peak increased until it reached twice the amount in the 18S RNA peak (data not shown). At 39 °C, the synthesis and transport of the 18S RNA seemed to be unimpaired, but little newly formed 28S RNA was found in the cytoplasm. Radioactivity in the 28S region was only about 5% of the 18S rRNA in the same cells grown at 39 °C. Similar results were obtained by Toniolo *et al.*<sup>13</sup> after 20 h observation. The reduced appearance of 28S rRNA

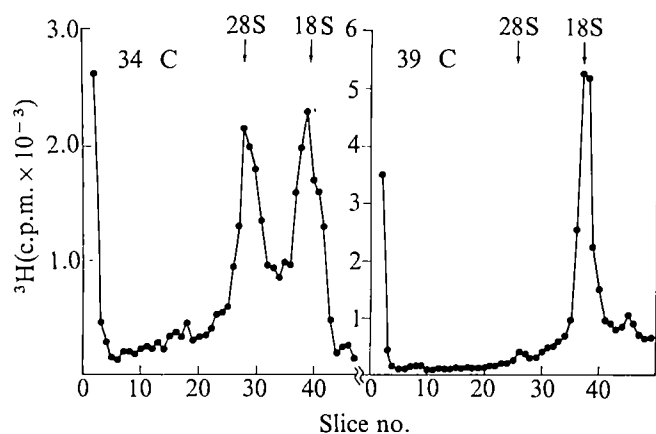


Fig. 1 Electrophoretic analysis of cytoplasmic RNA labelled during growth at 34 and 39 °C. Cultures were kept at 34 or 39 °C for 24 h and incubated for another 2 h in the presence of <sup>3</sup>H-uridine (1.0 µCi per ml medium, New England Nuclear). Cells were treated with 0.5% Triton X-100 in phosphate buffer (pH 7.4) containing 1.5 mM MgCl<sub>2</sub>. After 20 min, nuclei were centrifuged to a pellet, and the cytoplasmic and nuclear fractions were extracted three times with phenol. A sample of the aqueous phase was added directly to the gel slot and electrophoresed for 90 min in mixed polyacrylamide-Agarose gels<sup>14</sup>. Values represent c.p.m. per slice.

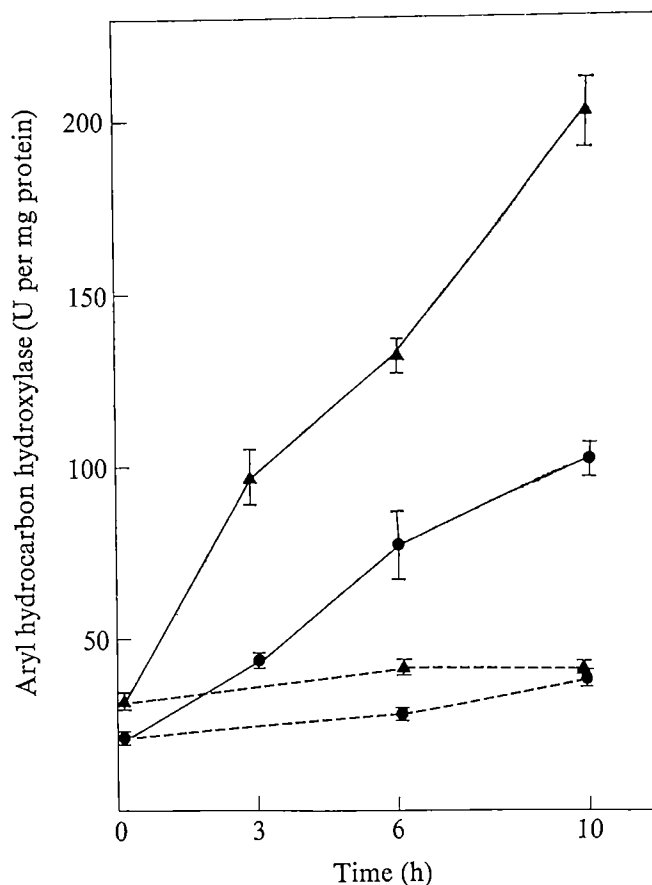


Fig. 2 Induction of aryl hydrocarbon hydroxylase in BHK-422E cells at 34 and 39 °C. Benz(a)anthracene (3 µg per ml medium) was added to cultures after 24 h of incubation at 34 and 39 °C. Cultures were further incubated at the two temperatures and at various times cells from two plates each were pooled. Aryl hydrocarbon hydroxylase activity was assayed by the procedure of Nebert and Gelboin<sup>7</sup> with minor modifications<sup>5</sup>. The incubation period was 60 min. A 2.0 ml sample of the organic layer was extracted into 1 ml of 1 N NaOH. One unit of aryl hydrocarbon hydroxylase catalyses in 1 min the formation of phenolic product with the fluorescence equivalent of 1 fmol of 3-hydroxybenzo(a)pyrene. Protein concentrations were determined by the method of Lowry *et al.*<sup>18</sup> with ribonuclease A as standard. Values represent the mean and range of duplicate determinations in the absence (----) and in the presence (—) of benz(a)anthracene. ▲, 39 °C; ●, 34 °C.

in the cytoplasm has been attributed to a defect in the processing of precursor 32S rRNA<sup>13</sup>. Electrophoretic analysis of nuclear fractions showed considerable radioactivity in the region of 32S RNA in extracts from cultures incubated at both 39 and 34 °C, but as in the case of cytoplasm, virtually no radioactivity appeared in the 28S region of cultures incubated at 39 °C (data not shown).

The time course of aryl hydrocarbon hydroxylase induction was then examined in the mutant BHK cells at temperatures permissive and non-permissive for 28S rRNA synthesis (Fig. 2). Cultures were set at the respective temperatures 24 h before addition of the inducer, benz(a)anthracene. Hydroxylase activity increased nearly linearly for 10 h at both temperatures. Constitutive hydroxylase activity and inducibility were higher at 39 than at 34 °C. The higher levels of hydroxylase activities at the non-permissive temperature might be an expression of a generally greater metabolic activity at the higher temperature.

The results suggest that aryl hydrocarbon hydroxylase induction occurs in the absence of *de novo* formation of 28S RNA. The ability of the mutant BHK cells to form 28S RNA is lost exponentially with a half time of less than 5 h in cells shifted from 34 to 39 °C (ref. 13). In view of the 24 h preincubation period at 39 °C and the relatively short half life of rRNA

**Table 1** Aryl hydrocarbon hydroxylase induction in BHK-422E cells during treatment with actinomycin D and cycloheximide

Group	Treatment*	Incubation period (h)†	Aryl hydrocarbon hydroxylase (U per mg protein)‡
1	Control medium	8	3.9±0.1
2	BA	8	40.3±1.0
3	BA+act D, 2 µg	8	6.5±0.3
4	BA+act D, 4 µg	8	8.3±0.3
5	BA+cycloheximide	8	2.3±0.1
6	BA+cycloheximide followed by BA+act D	3 5	145.3±5.0
7	BA+cycloheximide+act D followed by BA+act D	3 5	19.1±0.5

\*Cultures were treated with benz(a)anthracene (BA), 3 µg in 3 µl of dimethylsulphoxide (DMSO) per ml medium (BA, groups 2–7) or with 3 µl of DMSO (control medium, group 1). Cycloheximide was used at 10 µg ml<sup>-1</sup> (groups 5–7), actinomycin D (act D) at 1 µg ml<sup>-1</sup> (groups 6–8).

†Cultures of groups 6 and 7 were treated for an initial period of 3 h. The medium was then changed and cultures were treated for a further 5 h as indicated.

‡Mean and range of duplicate determinations.

in the nucleus<sup>14,15</sup>, it seems unlikely that significant amounts of unlabelled 28S rRNA are transferred to the cytoplasm. It is also unlikely that the small residual 28S rRNA production at 39 °C (Fig. 1 and ref. 13) has significance in the enzyme induction. This would imply that either 28S rRNA is produced in excess over that needed for translation of essential proteins at the permissive temperature, or that a specific induction-related 28S rRNA is preserved at the non-permissive temperature.

The requirement for RNA synthesis during aryl hydrocarbon hydroxylase induction in the mutant BHK cells at 39 °C is shown in Table 1: actinomycin D (2 or 4 µg ml<sup>-1</sup> medium) inhibited the increase in enzyme activity during 8 h (compare groups 1–4); after a block of protein synthesis in the presence of the inducer which then is followed by release of protein synthesis and a block of RNA synthesis, the enzyme activity increased more than 30-fold (group 6). This enhanced induction is largely suppressed when actinomycin D is present during the initial block of protein synthesis (group 7). The phenomenon of enhanced induction after a temporary block of protein synthesis is discussed elsewhere<sup>9,16</sup>. The results indicate that aryl hydrocarbon hydroxylase induction in BHK-422E cells at 39 °C, as in other cells in culture at 37 °C (refs 5, 9, 11 and 12), depends on a transient *de novo* RNA synthesis which may be independent of concomitant protein synthesis.

Enzyme induction in the virtual absence of 28S rRNA formation is interesting in two aspects. First, it has been suggested that the rate of rRNA synthesis, maturation and transfer have a role in adaptive changes of enzyme activities during cellular growth, differentiation and hormone action<sup>1,2</sup>. This is not supported by the results presented here, although rRNA requirements may differ between the highly selective, substrate-mediated induction of the hydroxylase and the broader regulatory processes affected by hormones. Second, rRNA synthesis and transfer have been implicated in the control of mRNA synthesis and expression<sup>3,4</sup>. Induction of aryl hydrocarbon hydroxylase clearly requires the synthesis of a specific RNA<sup>5,9,11</sup>. The effects of preferential inhibition of rRNA synthesis<sup>5</sup> or of heterogeneous RNA synthesis<sup>17</sup> during induction suggested the involvement of an RNA of the heterogeneous informational type. In agreement with earlier observations<sup>5</sup>, the results indicate that this induction-specific RNA is synthesised and transferred to the cytoplasm independently of concomitant 28S RNA formation and transfer. The results do not exclude the possibility that a nucleolar function is essential in the flow of information from the nucleus to the cytoplasm as postulated by Harris *et al.*<sup>3,4</sup>, but the processing and transfer of 28S RNA now can be eliminated from consideration in the search for this function.

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## Compact form of DNA induced by spermidine

RECENT studies have revealed that the packaging of DNA into phage heads is an ordered sequence of structural and biochemical events rather than a simple, spontaneous self-assembly of component molecules. In the assembly of T7 for example<sup>1</sup>, heads containing the products of genes 8–10 and 14–16 are apparently formed first. Gene 9 protein is removed from the heads before or during the incorporation of DNA. At least two other gene products which play no direct structural role are required for the maturation of the heads. A somewhat similar situation exists for phage λ, which has been shown to require ATP for the *in vitro* packaging of DNA into preformed heads (petite λ)<sup>2</sup>.

Apart from the mechanistic description of the specific processes of DNA packaging, there is the important question of whether these processes are facilitated by a thermodynamic driving force toward the compaction of DNA. Packaging *in vitro* DNA (that is, DNA in 0.2 M ionic strength with a small amount of buffer) without some generalised driving force would be so disadvantageous thermodynamically that it does not seem to be a feasible process. For example, T7 DNA may be represented by an effective ellipsoid<sup>3</sup> with a volume of ~10<sup>12</sup> Å<sup>3</sup> (ref. 3). This is roughly 10,000 times the volume of the T7 phage head. A compression of this magnitude would result in a considerable opposing pressure. In addition to this essentially entropic force, there would also be the work required to bring the negatively charged segments of DNA into close proximity to one another and the energy required to bend or fold the stiff DNA chains into a compact structure. If packaging were to take place with all of these opposing forces in full operation, not only would a great deal of free energy be required for the process, but the phage would be 'spring-loaded' to an increasing degree during the packaging and would expel the DNA spontaneously if there were any relaxation in the packaging mechanism. If by contrast phage particles are formed in an ambience in which these forces are overcome or strongly diminished, the agents responsible for the packaging would have the simpler role of selecting a proper mechanistic pathway for a process which is essentially spontaneous.

The notion that the free energy of the compacted form is lowered by its interaction with polyamines<sup>4</sup>,  $Mg^{2+}$ , (ref. 5) cationic polypeptides<sup>6</sup>, or other reagents has been in the literature for some time. Lerman *et al.*<sup>7</sup> have shown that another possible way of favouring the collapse of DNA is by raising the free energy of the expanded form. This is accomplished by the addition of high polymers which interact unfavourably with DNA. The condensed form is simultaneously stabilised by the presence of high salt concentrations. DNA condensed in this manner ( $\psi$  DNA) has also been studied by Evdokimov *et al.*<sup>8</sup> and by Laemmli *et al.*<sup>9</sup> and it seems to be well established that this treatment in sufficiently dilute solution converts single molecules of DNA into compact, dense particles.

It was our desire to see if the same effect could be achieved using materials which are known to exist in host bacterial cells, in particular, the polyamines. This idea is relatively old—for example an early investigation by Kaiser *et al.*<sup>4</sup> indicated that DNA in the presence of spermine is converted to a form which is protected from shear. The reason that the notion has not been pursued further presumably lies in the difficulty of studying such systems<sup>10</sup>. In terms of polymer theory<sup>11</sup>, conditions are required in which segmental attraction is strong (Flory-Fox parameter  $\alpha < 1$ ) and these are precisely the conditions which are well known to cause aggregation and precipitation. The preventative against this is very low concentration. Lerman's group<sup>12</sup> was able to demonstrate monomolecular collapse by the sedimentation of radioactive DNA which was converted into the  $\psi$  form in extremely dilute solution. In general, a physical method of observing the collapse is difficult to find since most techniques become inoperative at the dilutions required.

The technique selected for this investigation is streaming linear dichroism. A brief description of the apparatus has been published<sup>13</sup> and the details will be published soon<sup>14</sup>. The

procedure is the same as for streaming birefringence except that linear dichroism (LD) at 254 nm is used for finding the angle of isocline,  $\chi$ . The strong absorptive properties at 254 nm combined with stress modulation and lock-in amplification provides great sensitivity for the measurement of both  $\chi$  and the magnitude of the LD. Moreover, stream orientation is extremely sensitive to molecular dimensions. With this technique it is possible to make measurements on DNA where aggregation can be largely forestalled.

The effect of spermidine concentration on the orientational properties of DNA is shown in Fig. 1 which plots the isocline angle against gradient. In the conditions of the experiment (0.001 M NaCl, 0.001 M Na cacodylate, pH 6.6), there is an abrupt change in properties at about  $4.6 \times 10^{-5}$  M spermidine.

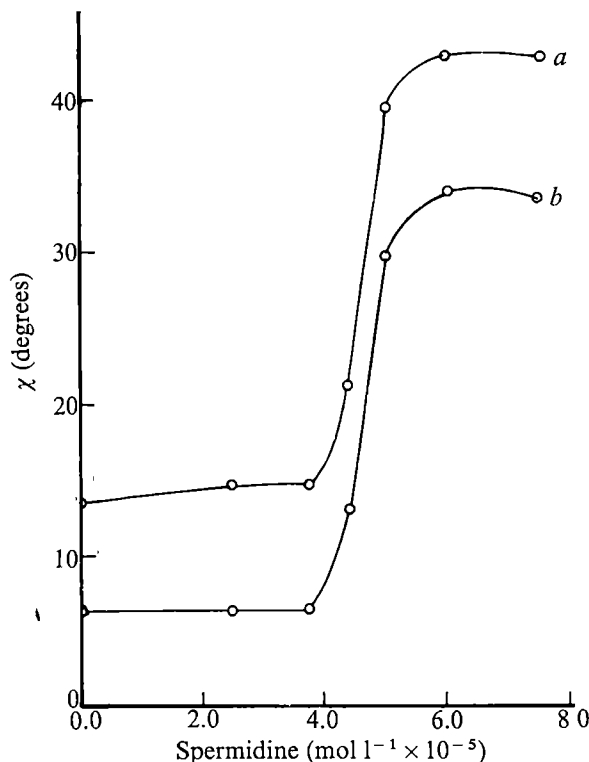
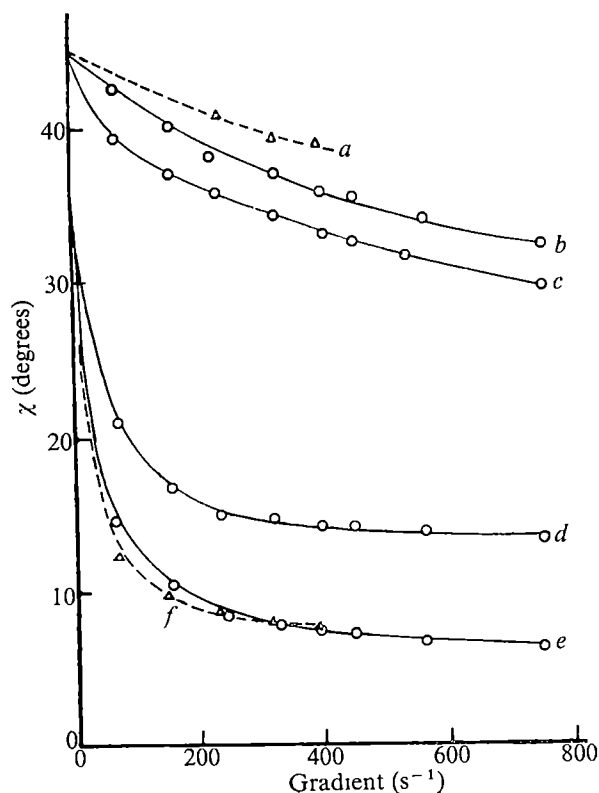
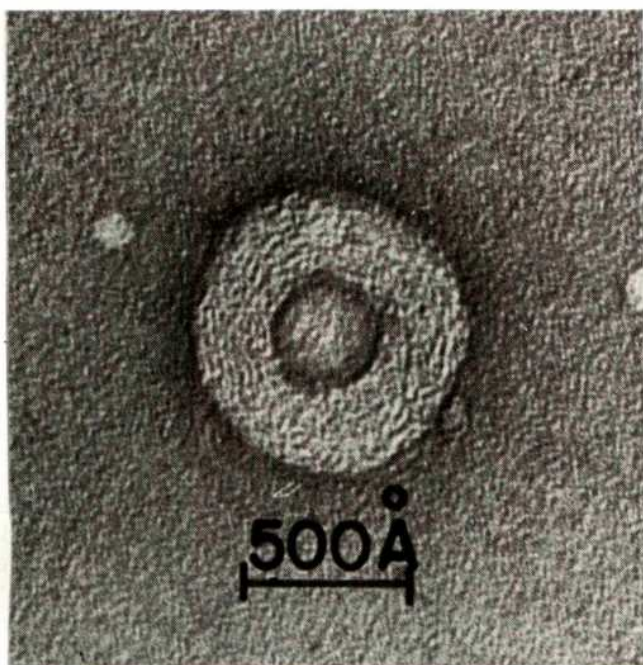


Fig. 2 Variation of isocline angle of T7 DNA with spermidine concentration at extreme values of gradient: a,  $74.2 \text{ s}^{-1}$ ; b,  $742 \text{ s}^{-1}$ . Conditions as in Fig. 1. DNA  $A_{260} = 0.1$ .



These results are indicative of a drastic cooperative change in size and shape. The effect is brought out more clearly in Fig. 2 which plots the angle of isocline against spermidine concentration for two fixed velocity gradients. The lower curves of Fig. 1 are typical for a long flexible molecule which is being simultaneously stretched and oriented by the hydrodynamic field. The upper curves are typical for a molecule of much smaller dimensions with a low degree of orientation in the hydrodynamic gradient. If the molecules were compacted into spheres, there would be no orientation and the LD would vanish. Most of the experiments in Figs 1 and 2 were carried out at a moderately low DNA concentration ( $A = 0.1$  in a 1-cm cell). In these conditions, accurate measurements may be made throughout the transition but control experiments with the ultracentrifuge indicate heterogeneity, presumably the result of aggregation. To demonstrate that aggregation is not a necessary part of the phenomenon, experiments were carried out in highly dilute solutions ( $A = 0.006$ ). These solutions showed evidence of aggregation only after a number of hours. A typical experiment on the effect of spermidine on such dilute solutions is shown in Fig. 1 ( $\Delta$ ). Data on the collapsed form are less accurate because of the combination of low concentration and low LD, although the disappearance of native extended DNA is clear cut.





**Fig. 3** Electron micrograph of spermidine-collapsed T7 DNA. Carbon-coated copper grids were coated with a monolayer of cytochrome *c* and glow-discharged. Grids were then floated for 10 min on a DNA-spermidine solution, touched to filter paper, floated on 1% uranyl acetate solution and blotted dry. Micrographs were obtained with a Phillips EM300 microscope.

Once the conditions for molecular collapse were established in the LD experiments, a number of ancillary experiments were performed to throw light on the structure of the collapsed form. Figure 3 shows an electron micrograph of a representative particle of DNA ( $A = 0.006$ ) in the presence of  $10^{-4}$  M spermidine in the same conditions of salt and pH as noted for Fig. 1. We consider the general size and shape of these particles to be more significant than the specific morphology since the latter is sensitive to sample preparation. For example, with uranyl acetate staining approximately 90% of the DNA molecules have the morphology of Fig. 3, whereas with unstained, shadowed preparations the molecules have approximately the same size and shape but only 10% have a central cavity. Note that Richards *et al.*<sup>15</sup> have reported the presence of hollow disks in electron micrographs of ruptured T7 phage.

Circular dichroism studies on the collapsed form yielded curves which differ only in negligible details from the B-form curves which are obtained before collapse. This is in contrast to the CD of the  $\psi$  form of DNA which is drastically different from the B form, and to the CD spectra of whole phages T5 and T7 which are distorted from the B form. This distortion has been attributed to the presence of C form DNA<sup>16</sup>.

Studies of the helix-coil transition of the collapsed DNA-spermidine complex show that the transition is shifted to a higher temperature and is considerably sharper than that of unperturbed DNA.

Experiments with other polyamines revealed that DNA is also collapsed by spermine at significantly lower concentrations than those required for spermidine. A description of these studies plus a more complete interpretation of the LD and electron micrograph experiments will be reported later.

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## Evidence for a major conformational change of coat protein in assembly of fl bacteriophage

RECENT structural studies<sup>1,2</sup> indicate that the DNA of filamentous bacteriophages is enclosed in a sheath of  $\alpha$ -helical coat protein. In the course of phage biosynthesis this protein is tightly bound to the membrane of the host (*Escherichia coli*)<sup>3,4</sup>. We present here the results of circular dichroism (CD) studies strongly suggesting that the protein has a quite different conformation in its membrane-bound state, so that a major conformational change must accompany incorporation into the virus.

Methods for the preparation of fl and for the purification of coat protein have been described previously<sup>5</sup>. Accurate concentration measurements are necessary for the quantitation of CD spectra. These were made spectrophotometrically, using the following  $A$  values, based on dry weight determinations: for whole virus,  $A_{260} = 3.78$  or  $A_{268} = 3.95$ ; for purified coat protein,  $A_{260} = 1.65$ . The values given apply to a solution containing 1 mg ml<sup>-1</sup> and a light path of 1 cm. In the experiments using phosphatidylcholine vesicles, where light scattering interfered with spectrophotometric measurements, coat protein containing radioactive tyrosine and lysine was used, specific activity was determined relative to absorbance in the intact phage, and concentration in the vesicles was obtained on the basis of radioactive counts. CD spectra were measured using a Cary 60 spectropolarimeter with CD attachment.

Figure 1 shows the CD spectrum in the peptide absorption region for intact virus and for virus dissociated by 30 mM deoxycholate<sup>6</sup>, but without separation of the components. The possible contribution from DNA to these spectra must be small, both because the DNA content of the virus is small and because the magnitude of the CD spectrum of DNA is low in this spectral range, regardless of conformation<sup>6-8</sup>. The difference between the curves of Fig. 1 must therefore arise predominantly from a conformational change of the viral protein, more than 98% of which is the viral coat protein.

Mean residue ellipticities of the viral protein obtained from Fig. 1 (assuming that DNA makes no contribution at all) are shown in Fig. 2. A protein content of 88.5% (determined in this laboratory, but agreeing with previous measurements<sup>9</sup>) was used, together with a mean residue molecular weight of 105, calculated from the amino acid composition of the protein. Fig. 2 also shows the CD spectra of chromatographically purified coat protein in 30 mM deoxycholate, of coat protein precipitated by removal of deoxycholate by ultrafiltration and redissolved in 30 mM deoxycholate, and of coat protein similarly precipitated and redissolved in 5 mM oleyl lysophosphatidyl-



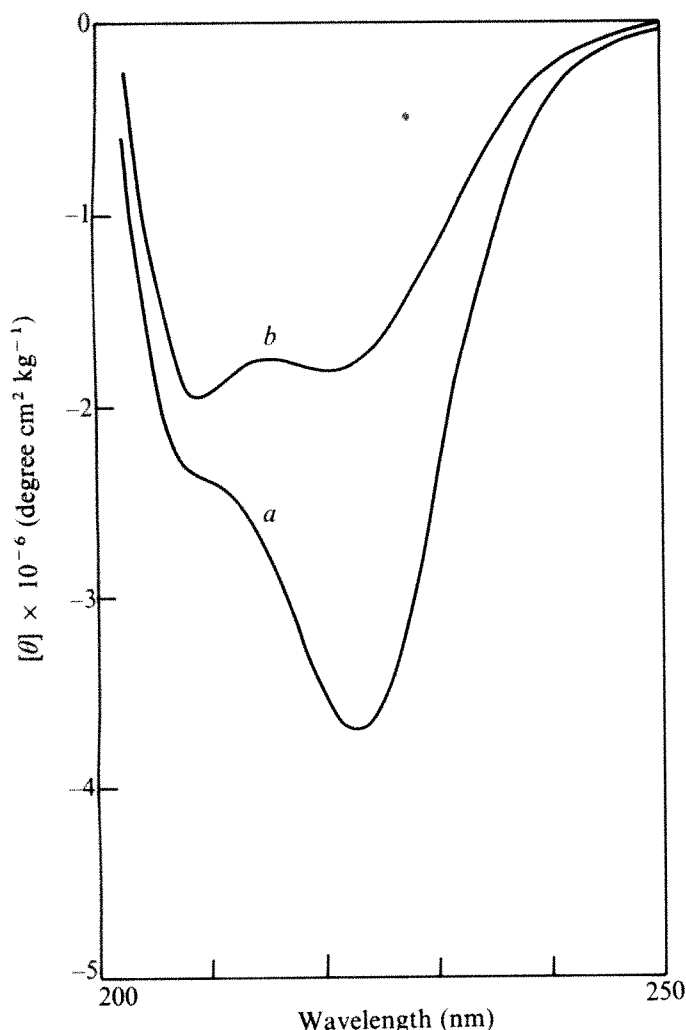


Fig. 1 Circular dichroism (per kg virus) of intact virus in 0.1 M NaCl (a) and after dissociation by 30 mM deoxycholate (b).

choline. (Spectra similar to curves *d* and *e* were obtained in two other detergents as well and in two other micelle-forming lysolipids.) Figure 2 also shows the CD spectrum of coat protein incorporated into egg yolk phosphatidylcholine vesicles. Phage in  $\text{CHCl}_3$ -saturated aqueous solution (where the CD spectrum is identical to that of intact phage) was deposited on the walls of a conical centrifuge tube by evaporation of solvent with dry  $\text{N}_2$ . Egg yolk phosphatidylcholine dissolved in  $\text{CHCl}_3$  was deposited in the same way. Aqueous buffer was added and vesicles were formed by sonication as described by Huang<sup>10</sup> and chromatographed on Sepharose 4B. The coat protein (radioactively labelled) was found to have been separated from viral DNA and eluted with the phospholipid vesicles. The molar ratio of lipid phosphorus to protein in the combined peak was 80. The protein was accessible to proteolytic enzymes, proving that it was not simply trapped in the vesicles during sonication.

The CD spectrum for the coat protein in the intact virus is unlike any known spectrum for a protein or polypeptide in simple aqueous solutions<sup>11,12</sup>: the peak wavelengths are appropriate for an  $\alpha$ -helical chain, but the unequal intensities of the bands at 208 and 222 nm are not. Similar unequal intensities, however, have been frequently observed for proteins in particulate suspension<sup>13,14</sup> and they have been theoretically explained on the basis of bunching of the absorbing chromophores into a small space, leading to local concentrations far in excess of the average concentra-

tion of the protein or polypeptide in the solution as a whole<sup>13</sup>. It is entirely appropriate to observe this effect in the virus, in which over 2,000 protein molecules are contained in a single particle. Since the effect leads to a diminution of intensity, always more pronounced at 208 than at 222 nm, the observed spectrum clearly falls within the range expected for proteins or polypeptides with an  $\alpha$ -helical content of 100% (refs 11–13). Our result is thus consistent with the X-ray data cited earlier. It also agrees with previous estimates based on optical parameters<sup>15,16</sup>.

The CD spectra of amphiphile-solubilised protein are all similar and differ greatly from the spectrum in the intact virus. The helical content is less than 50%. No increase in negative CD is observed below 210 nm, indicating<sup>11</sup> that no significant portion of the protein has been converted to a structureless random coil. Part of the non-helical portion of the structure could be in the  $\beta$ -conformation or some twisted variant thereof. If so, it is likely that the hydrophobic amino acid residues (residues 21–39)<sup>17</sup> are involved and this might explain why the coat protein is always in a dimeric state in those detergents in which molecular weight measurements have made<sup>5</sup>, since  $\beta$  structures require interchain hydrogen bonds for stabilisation. Estimates of the location of probable helical segments were made for us

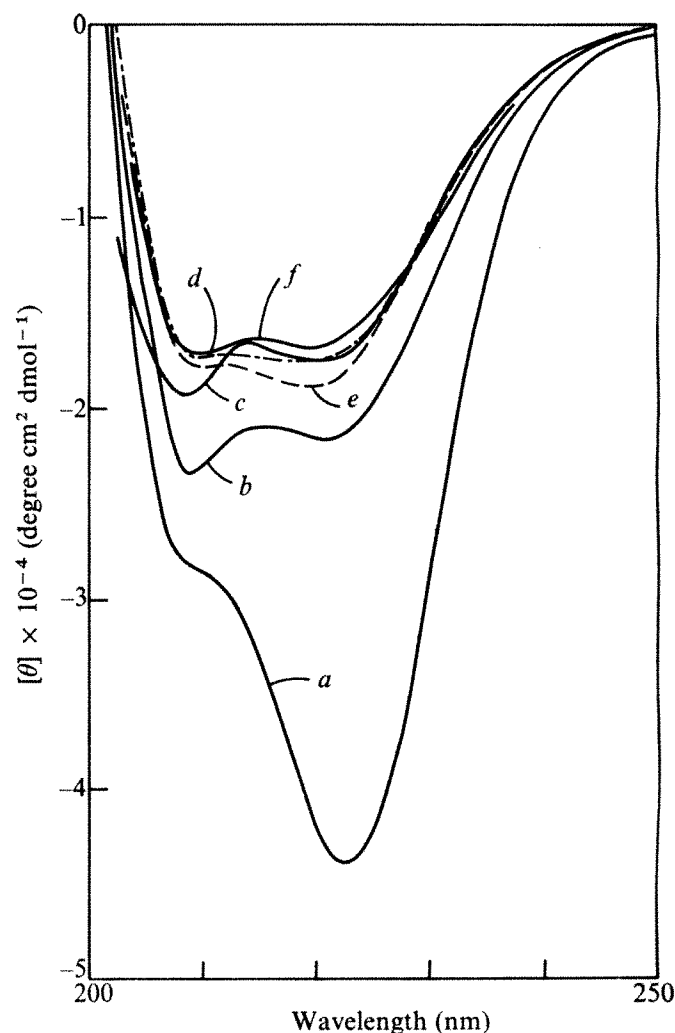


Fig. 2 Mean residue ellipticity of coat protein. *a*, In intact virus, from Fig. 1 assuming no contribution from DNA; *b*, in deoxycholate micelles (30 mM), from Fig. 1 with same assumption; *c*, purified protein in 30 mM deoxycholate; *d*, purified protein redissolved in 30 mM deoxycholate after precipitation; *e*, purified protein in oleyl lysophosphatidylcholine micelles (5 mM) after precipitation; *f*, purified protein in egg yolk phosphatidylcholine vesicles, prepared as described in the text.

by Drs Chou and Fasman and by Drs Krigbaum and Knutton, using the methods they have described previously<sup>18,19</sup>. Both groups predict helical segments near both chain termini and assign a probable  $\beta$ -pleated structure to residues 26–37 and 27–36, respectively. (The fd coat protein sequence<sup>17</sup> was used for this analysis, but we have found fd and fl to be identical in amino acid composition and the pattern of proteolytic fragments<sup>20</sup>.)

If we make the reasonable assumption that the similar spectra observed in detergent micelles, phospholipid micelles and phospholipid vesicles also represent the state of the coat protein when bound to the membrane in the host bacterium, and take into account the proteolytic digestion data presented previously<sup>20</sup>, we are led to the conclusion that the membrane-bound state has the central portion of the coat protein polypeptide inserted into the hydrophobic core of the membrane, very likely in a  $\beta$  conformation. A major structural change must thus occur when the protein is removed from the membrane and incorporated into newly-assembled phage particles.

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## Hyper-repressible operator-type mutant in sulphate permease gene of *Aspergillus nidulans*

SINCE the pioneering work on regulation of the *lac* operon in *Escherichia coli*<sup>1</sup> a great number of operator mutations have been described. In eukaryotic organisms, however, only a few mutations affecting *cis*-acting regulatory element (some of which can be formally interpreted as operator mutants) have been reported<sup>2–6</sup>. The term 'operator' in the case of eukaryotes is used in an operational sense as it is not known whether it describes a molecular organisation similar to that found in bacteria. This report describes a mutant of *Aspergillus nidulans* altered at a site closely linked to the *sB* gene for sulphate permease<sup>7</sup>. This mutation causes a hypersensitivity of this gene to repression by sulphur amino acids.

Sulphate permease in *A. nidulans* is completely repressed if the mycelium is grown in the presence of cysteine, homocysteine or methionine. Toxicity of chromate, a substrate to the sulphate permease, is therefore easily reversed by the addition of methionine to the growth medium. More than 100 mutants resistant to chromate have been isolated in *A. nidulans*<sup>8</sup>, all of which were allelic, both in heterokaryons and diploids, to the previously known mutant *sB3*, indicating that the mutations affect sulphate permease<sup>8</sup>. One of these mutants, *sB<sub>90</sub>*, was particularly interesting as, unlike other mutants, it showed a normal growth rate on minimal medium. Chromate (1 mM) inhibited growth of this mutant but 0.03 mM L-methionine was sufficient to reverse chromate toxicity, whereas 5 mM L-methionine was necessary in the case of the wild type. This suggested that the mutant takes up less chromate than the wild type because of altered specificity of sulphate permease or its hypersensitivity to repression. Table 1 supports the second alternative. When the mutant and the wild type were grown in sulphur-free medium (there was enough sulphur in the conidia used for inoculation to ensure growth for 12–16 h), the rate of sulphate uptake in both strains was very similar (see also Table 2).  $K_m$  for sulphate was  $5.0 \times 10^{-5}$  M and  $4.0 \times 10^{-5}$  M, respectively, for wild-type and *sB<sub>90</sub>* strains. A similar inhibition of sulphate uptake by chromate in both strains was observed:  $K_i = 3.3 \times 10^{-5}$  M for wild type and  $K_i = 3.4 \times 10^{-5}$  M for the mutant *sB<sub>90</sub>*. These data indicate that the mutation *sB<sub>90</sub>* does not alter the sulphate permease itself, but rather the regulation of its synthesis. On the other hand, in more than 700 progeny examined from the cross *sB<sub>90</sub> × sB3* no wild-type segregants (which would be chromate-sensitive) were found. The *sB<sub>90</sub>* site must therefore be situated within or very close to the *sB* locus. The simplest hypothesis would be that *sB<sub>90</sub>* affects a regulatory site involved in the repression of sulphate permease, so that its affinity to a regulatory molecule is increased.

Table 1 Effect of L-methionine in growth medium on level of sulphate permease in *sB<sub>90</sub>* and wild-type strains

Strains	Sulphate uptake (nmol per min per mg dry weight)		MM—S
	Control MM—S	MM—S + 0.3 mM L-methionine*	MM—S + meth
<i>sB<sub>90</sub></i>	5.95	0.47	12.7
Wild type	7.62	2.38	3.2

Mycelia were grown and sulphate uptake assayed as described previously<sup>10</sup>. MM—S, minimal medium without sulphur source.

\* Minimal concentration that enables strain *sB<sub>90</sub>* to grow on 1 mM chromate (Table 2).

To test this hypothesis we used a recessive regulatory mutant *suAmeth* in which several enzymes of methionine synthesis including sulphate permease are constitutive<sup>9</sup>. The character of this mutation strongly suggests that this gene is responsible for the synthesis of a repressor-like molecule involved in a negative control system. Due to the constitutive formation of sulphate permease the strain *suAmeth* is extremely sensitive to chromate even in the presence of a high concentration of methionine (Table 2). If the *sB<sub>90</sub>* mutation causes an increased affinity to the regulatory molecule one would expect to find the mutation *suAmeth* epistatic to *sB<sub>90</sub>*, as without active repressor the latter mutation cannot be manifested. This was the case. As shown in Table 2 the double mutant *sB<sub>90</sub>suAmeth* is equally sensitive to chromate as is *suAmeth* alone. As expected, strain *sB3suAmeth* displays the phenotype of *sB3* because even constitutive synthesis of impaired sulphate permease does not lead to an increased chromate uptake. Experiments on sulphate uptake (Table 3) confirm the results of growth tests. Strain *sB<sub>90</sub>suAmeth* shows a constitutive synthesis of sulphate permease. Thus, the introduction of the *suAmeth* mutation to strain *sB<sub>90</sub>* results in the changeover from hyper-repressibility to constitutive synthesis

**Table 2** Effect of *suAmeth* mutation on growth of *sB3* and *sB<sub>90</sub>* strains on L-methionine (10 mM) and various concentration of chromate

Chromate (mM)	Wide type	<i>sB3</i>	<i>sB<sub>90</sub></i>	Strains		
				<i>suAmeth</i>	<i>sB3suAmeth</i>	<i>sB<sub>90</sub>suAmeth</i>
0.03	+	+	+	+	+	+
0.05	+	+	+	±	+	±
0.06	+	+	+	—	+	—
1.00	+	+	+	—	+	—
1.50	±	+	+	—	+	—
2.00	—	+	+	—	+	—

Growth tests on solid medium (24 h at 37 °C) were carried out as described previously<sup>8</sup>. Growth symbols: +, normal growth; ±, weak growth; —, no growth.

**Table 3** Sulphate uptake by *sB3*, *sB<sub>90</sub>*, *suAmeth* and double mutant strains grown with and without L-methionine

Strain	Sulphate uptake (nmol per min per mg dry weight)	
	MM—S	MM—S + L-methionine (10 mM)
Wild type	10.65	0.00
<i>sB3</i>	0.00	0.00
<i>sB<sub>90</sub></i>	10.18	0.00
<i>suAmeth</i>	11.57	8.33
<i>sB3suAmeth</i>	0.00	0.00
<i>sB<sub>90</sub>suAmeth</i>	12.00	10.13

Culture conditions and sulphate uptake assays were as described previously<sup>10</sup>. Djenkolic acid was added to the *sB3* and *sB3suAmeth* cultures as a sulphur source. It does not cause repression of sulphate permease in the wild type.

**Table 4** Activity of ATP sulphurylase and homocysteine synthase in *sB3*, *sB<sub>90</sub>*, *suAmeth* and double mutant strains

Strain	Activity (nmol per min per mg protein)	
	ATP sulphurylase	Homocysteine synthase
Wild type	71.8	35.6
<i>sB3</i>	69.3	32.8
<i>sB<sub>90</sub></i>	72.4	48.5
<i>suAmeth</i>	116.8	120.0
<i>sB3suAmeth</i>	152.0	138.5
<i>sB<sub>90</sub>suAmeth</i>	130.0	154.5

Mycelia for enzyme assays grown, extracted and assayed as described previously<sup>10</sup>.

of the sulphate permease; these data confirm both the operator-type nature of the *sB<sub>90</sub>* mutation and the regulatory role of the *suAmeth* gene product.

One of the most characteristic features of an operator mutation is that it is *cis*-acting. The interpretation of a *cis-trans* test is straightforward in the case of an operator-constitutive mutant which is dominant, but not in the case of a recessive mutant like *sB<sub>90</sub>*. Diploid *sB<sub>90</sub>/sB<sup>+</sup>* was sensitive to chromate indicating the recessive character of the mutation.

We have tested two other enzymes that are controlled by the *suAmeth* gene<sup>9</sup>—ATP sulphurylase and homocysteine synthase (Table 4). No significant differences in the level of these enzymes were observed between *sB3*, *sB<sub>90</sub>* and wild-type strains. Both enzymes were derepressed in the *sB3suAmeth* and *sB<sub>90</sub>suAmeth* strains. This is additional evidence that the mutation *sB<sub>90</sub>* affects only the regulation of sulphate permease.

All operator or operator-type mutants described so far in fungi have been selected as having increased activity over that of the wild type, at least in some growth conditions. Recognition of *cis*-acting regulatory mutations in control regions adjacent to structural genes which lead to reduced activity are much more difficult to recognise as they cannot be readily distinguished from mutations within structural genes themselves. The *sB<sub>90</sub>* mutation, however, is a clear indication that such mutations do exist.

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## α and β Chains of the major haemoglobin and a note on the minor component of *Tarsius*

THE position of *Tarsius* (the tarsier) in the evolution of the primates has long been controversial. Gadow<sup>1</sup> placed it in a separate suborder, but Pocock<sup>2</sup> allied it with the monkeys, apes and man in the Haplorhini, assigning the lemurs, lorisooids and tree shrews to the Strepsirhini. This was accepted by Hill<sup>3</sup> but Simpson<sup>4</sup> divided the primates into two suborders, Anthropoidea and Prosimii, placing *Tarsius* in the latter together with lemurs, lorisooids and tree shrews, though as a separate infraorder. The problem arises

because of resemblances between *Tarsius* and the anthropoids. These include the absence of a rhinarium, a retinal structure similar to the anthropoid fovea and a haemochorial placenta, compared with the epitheliochorial form of prosimians, *Tarsius* is often regarded as a 'living fossil', implying that little evolution has taken place since its divergence from the other primates. The problem may be solved with the aid of data on the primary structure of mammalian proteins<sup>6</sup>: there is sufficient evidence about haemoglobin, for example, to construct a hypothetical ancestral primate sequence<sup>5-7</sup>. Using two samples of blood from *T. bancanus* collected during the past 3 yr, we have determined the  $\alpha$  and  $\beta$  Hb chain sequences and found that, at the molecular level, *Tarsius* is not a 'living fossil' and that the most parsimonious globin trees cluster *Tarsius* with the anthropoids and not the prosimians, lending support to the Haplorhini-Strepsirhini classification<sup>2,3</sup>.

Both our samples had two haemoglobins on starch-gel electrophoresis at pH 8.6 (Tris-EDTA-borate)<sup>8</sup>; the major and minor components had mobilities identical with those of human HbA ( $\alpha_2\beta_2$ ) and HbA<sub>2</sub> ( $\alpha_2\delta_2$ ) respectively. The two components were separated by ion exchange chromatography on DEAE-Sephadex<sup>9</sup> and absorbances at 540 nm of the pooled fractions showed the minor component to be 18% of the total haemoglobin in each case. The major component was used for sequence studies. Globin was prepared by acid-acetone precipitation<sup>10</sup> and the chains were separated<sup>11</sup>. The amino acid sequences of the  $\alpha$  and  $\beta$  chains were determined after tryptic digestion of the aminoethylated chains. The evidence for the sequences will be given in more detail elsewhere.

The amino acid sequence of the tarsier  $\alpha$  chain differed from the human  $\alpha$  chain in eleven positions and the  $\beta$  chain had fifteen differences from the corresponding human chain. Adequate evidence was obtained for both sequences except for two short regions which are based on amino acid compositions and homology with other globin chains. These are residues 129-136 of the  $\alpha$  chain (from a chymotryptic peptide) and residues 67-75 of the  $\beta$  chain, the sequence of which was postulated after amino acid analysis of two thermolytic peptides.

The available data on primate  $\alpha$  and  $\beta$  chains<sup>12-21</sup> is summarised in Tables 1 and 2 respectively. Only residues that are variable among primates are shown together with the hypothetical primate and anthropoid ancestral residues constructed by Goodman *et al.*<sup>7</sup> using the parsimony principle. The construction of these ancestral residues was not biased by the data for the tarsier. Only sequences for which there is a reasonable amount of evidence have been considered.

There are several changes from the ancestral sequence in both chains which, among primates, are unique to the tarsier ( $\alpha$  15, 53, 68, 73, 129;  $\beta$  6, 19, 21, 52, 58, 69, 73, 75) and certainly do not give the impression that tarsier is a

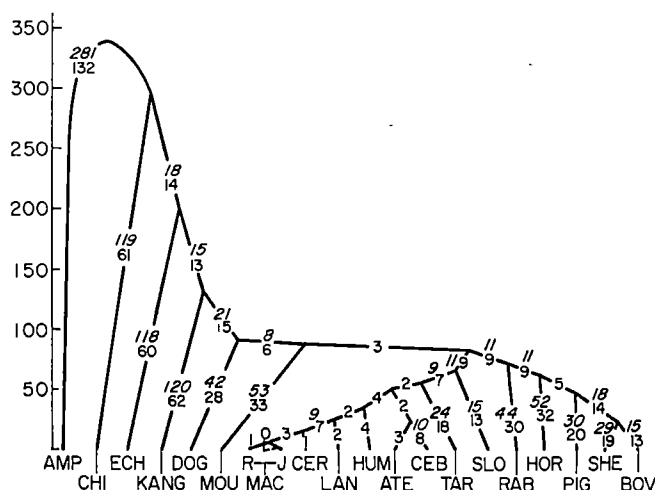


Fig. 1 Parsimony tree requiring 664 nucleotide replacements for 20 taxa on using a combined  $\alpha$ - and  $\beta$ -globin alignment. Link lengths are the numbers of nucleotide replacements between adjacent ancestral and descendant sequences; italicised numbers are link lengths corrected for superimposed replacements by the augmentation algorithm of Moore and Goodman<sup>6,7</sup>. The ordinate scale, in millions of years, is inferred from fossil evidence on ancestral splitting times of the taxa represented by the sequences. The taxa are: AMP (amphibian, represented by newt  $\alpha$  and frog  $\beta$ ), CHI (chicken), ECH (echidna), KANG (kangaroo), DOG (dog), MOU (mouse), R-MAC (rhesus macaque), J-MAC (Japanese macaque), CER (*Cercopithecus*), LAN (langur or *Presbytis*), HUM (human), ATE (*Ateles*), CEB (*Cebus*), TAR (*Tarsius*), SLO (slow loris or *Nycticebus*), RAB (rabbit), HOR (horse), PIG (pig), SHE (sheep), and BOV (bovine). The  $\alpha$ - and  $\beta$ -globin sequences from these animals are referenced in Beard and Goodman<sup>26</sup>.

'living fossil' at the molecular level. Most of the residues at which the tarsier sequences are unique among primates do not have a defined functional role<sup>22,23</sup>. One change in the  $\alpha$  chain, a Val instead of Leu at position 129 (H12), or position 136 (H19), is at the  $\alpha$ -haem contact. In the  $\beta$  chain, position 75 (E19) is internal and helps stabilise the tertiary structure. The Met found in tarsier has also been found in sheep  $\beta$  chain.

The original aim of the work on tarsier haemoglobin was to acquire sequence information relevant to the animal's phylogenetic position. If the ancestral primate sequence is correct it may be possible, by examining the known sequences, to find changes common only to tarsier and prosimians or to tarsier and the anthropoids. But if tarsier diverged from the anthropoid branch very soon after the prosimians, there may not have been time for substitutions to occur which would be inherited by both lineages. For the discussion which follows only those globins listed in Tables 1 and 2 have been considered.

Table 1 Variable residues in primate  $\alpha$ -globin chains

Residue no.	8	12	15	19	21	23	53	57	67	68	71	73	78	111	113	116	129
Primate ancestor*	Thr	Ala	Gly	Gly	Ala	Asp	Ala	Ala	Thr	Asn	Ala	Val	Ser	Ser	His	Asp	Leu
Anthropoid ancestor*	—	—	—	—	—	—	—	Gly	—	—	—	—	Asn	Ala	—	—	—
<i>Nycticebus</i>	—	—	Glu	Ser	—	—	—	—	—	—	Ser	—	—	Cys	—	—	—
<i>Tarsius</i>	—	—	Asp	—	—	—	Ser	Gly	—	Thr	Gly	Ile	Asn	Cys	—	—	Val
<i>Ateles</i>	Ser	—	—	—	—	—	—	Gly	—	—	—	—	Asn	Ala	—	—	—
<i>Cebus</i>	—	Thr	—	—	—	—	—	Gly	Ser	—	—	—	Asn	Ala	—	—	—
<i>Cercopithecus</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	His	Ala	Leu	Glu	—
<i>M. mulatta</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	Asn	Ala	Leu	Glu	—
<i>M. fuscata</i>	Ser	—	—	—	—	Glu	—	Gly	—	Leu	Gly	—	Asn	Ala	Leu	Glu	—
<i>Presbytis</i>	—	—	—	—	Gly	Glu	—	Gly	—	—	—	—	His	Ala	Leu	Glu	—
<i>Homo</i>	—	—	—	Ala	—	Glu	—	Gly	—	—	—	—	Asn	Ala	Leu	Glu	—

Residues that are variable among primates and differ from the primate ancestor are shown. The sequences considered are those of *Nycticebus coucang*<sup>12</sup>, *Ateles geoffroyi*<sup>13</sup>, *Cebus apella*<sup>14</sup>, *Cercopithecus aethiops*<sup>15</sup>, *Macaca mulatta*<sup>16</sup>, *Macaca fuscata*<sup>17</sup>, *Presbytis entellus*<sup>18</sup>, and human (*Homo*)<sup>19,20</sup>  $\alpha$  globins.

\* From the parsimony construction of Goodman *et al.*<sup>7</sup> (provided by Professor M. Goodman); these constructions also include the following alternative parsimony solutions at positions 23 (primate Glu, anthropoid Glu) and 111 (Ala, Ala). With these alternatives, however, inclusion of the tarsier sequence would add more mutations to the tree than with the solution used above.



Table 2 Variable residues in primate  $\beta$ -globin chains

Residue no.	5	6	9	13	19	21	22	33	43	50	52	56	58	69	73	75	76	87	104	112	121	125	126	128	139
Primate ancestor	Ala	Glu	Ser	Ala	Asn	Asp	Glu	Val	Glu	Ser	Asp	Gly	Pro	Gly	Asp	Leu	Asn	Lys	Arg	Phe	Glu	Gln	Val	Ala	Asn
Anthropoid ancestor	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Cys	—	—	—	—	—
<i>Nycticebus</i>	Gly	—	—	—	—	—	Asp	—	—	—	Ser	—	—	Ser	—	—	—	—	—	Val	Asp	—	—	Ser	—
<i>Tarsius</i>	Asp	Ala	—	—	Asp	Glu	Asp	—	Asp	Thr	Ala	—	Ala	Asn	Glu	Met	Ala	—	—	Cys	—	—	—	—	Thr
<i>Ateles</i>	Gly	—	—	—	—	—	—	—	—	Thr	—	Ser	—	—	—	—	Ala	Gln	—	Cys	—	—	Leu	—	—
<i>Cebus</i>	—	—	—	—	—	—	—	—	—	Thr	—	Asn	—	—	—	—	Thr	Gln	—	Cys	—	—	—	—	Thr
<i>Cercopithecus</i>	Pro	—	Thr	—	—	—	—	—	—	—	—	—	—	—	—	—	Ala	Gln	Lys	Cys	—	—	—	—	—
<i>M. mulatta</i>	Pro	—	Asn	Thr	—	—	—	Leu	—	—	—	—	—	—	—	—	—	Gln	Lys	Cys	—	—	—	—	—
<i>M. fuscata</i>	Pro	—	Asn	Thr	—	—	—	—	—	—	—	—	—	—	—	—	—	Gln	Lys	Cys	—	—	—	—	—
<i>Presbytis</i>	Pro	—	Ala	—	—	—	—	—	—	—	—	—	—	—	—	—	Ala	Gln	—	Cys	—	—	—	—	—
<i>Homo</i>	Pro	—	—	—	—	—	—	—	—	Thr	—	—	—	—	—	—	Ala	Thr	—	Cys	—	Pro	—	—	—

Residues which are variable among primates and differ from the primate ancestor are shown. The sequences considered are those of *Nycticebus coucang*<sup>9</sup>, *Ateles geoffroyi*<sup>13,21</sup>, *Cebus apella*<sup>14</sup>, *Cercopithecus aethiops*<sup>15</sup>, *Macaca mulatta*<sup>16</sup>, *Macaca fuscata*<sup>17</sup>, *Presbytis entellus*<sup>18</sup>, and human (*Homo*)<sup>19</sup>  $\beta$  globins.

\* From the parsimony construction of Goodman *et al.*<sup>7</sup> (provided by Professor M. Goodman); these constructions also include the following alternative parsimony solutions at positions 9 (primate Ala, anthropoid Ala); 50 (Ser, Ser), 52 (Asn, Asp), 69 (Ala, Gly), 87 (Lys, Gln), 112 (Ile, Cys and Val, Cys). With any of these alternatives, however, inclusion of the tarsier sequence would add more mutations to the tree than the solution used except at  $\beta$  9 and  $\beta$  87 where the alternatives could be used equally well as the anthropoid ancestors.

Unfortunately this selection only includes one prosimian, *Nycticebus*, although several anthropoids are known. Sequences have been reported (as unpublished data<sup>24,25</sup>) for certain other prosimians but adequate evidence has not been published.

In the  $\alpha$  chains shown in Table 1 there have been several changes from the ancestral sequence which could provide useful information. There is one residue which only *Tarsius* and *Nycticebus* have in common among the primates, that is Cys at position 111 instead of the ancestral Ser. At position 15 there is also evidence for a common ancestor of *Tarsius* and *Nycticebus*. On the other hand, there are two positions at which *Nycticebus* retains the ancestral sequence while tarsier and most of the anthropoids share a common change (57—Ala to Gly; 78—Ser to Asn). Both are single-base changes.

If one considers the  $\beta$  globins in Table 2, there is one position (22) at which tarsier and *Nycticebus* share a common change from the ancestral sequence. Also at positions 52 and 69, fewer mutations need have occurred if they shared a common ancestor after diverging from the anthropoids. At position 112, however, a Cys is present in tarsier and the anthropoids instead of the ancestral Phe, while *Nycticebus* has a Val residue at this position. Also at positions 50 and 76 *Nycticebus* has retained the ancestral sequence while tarsier and several anthropoids have acquired the same change. Thus, with respect to both  $\alpha$  and  $\beta$ -chain data, the hypothetical ancestral residues constructed independently of tarsier fail to indicate whether *Tarsius* is closer phylogenetically to Anthropoidea or to Prosimii.

Since the tarsier sequence might contain information needed for a closer approximation to the ancestral sequence, Beard and Goodman<sup>26</sup> included it in a combined  $\alpha$ - and  $\beta$ -globin alignment and used a branch-swapping procedure<sup>7</sup> to search extensively for the most parsimonious tree. In the several equally parsimonious alternatives found, tarsier was always closer to the Anthropoidea than to the slow loris, which supports the inclusion of the tarsier in a subdivision of the primates, Haplorhini<sup>2,3</sup>. Of these trees, that in Fig. 1 conforms closely to traditional evidence on the phylogeny of placental mammals. But more prosimian species need to be included to test adequately the conclusion and only two additional mutations would be added to the path length of the tree if tarsier and slow loris still shared a common ancestor (as prosimians) after the divergence of the Anthropoidea from prosimian primates. On the other hand, six additional mutations would be added if the positions of tarsier and slow loris were reversed. Moreover, if the representation of tarsier's phylogenetic position in Fig. 1 is correct, it is notable that the ancestral haplorhine node is closer to the ancestral anthropoid node than to the ancestral primate node. It should be noted that tarsier diverges more from the haplorhine ancestor than do most of the Anthropoidea. This clearly supports the

impression (see above) that at the molecular level, the tarsier is no 'living fossil'.

Finally, we wish to add a note on the minor component of tarsier haemoglobin. Minor components with a  $\delta$  chain instead of a  $\beta$  chain exist in man, the apes and New World monkeys but not Old World monkeys. Following the observation of a minor haemoglobin in tarsier with A<sub>2</sub>-like mobility<sup>8</sup> it has been suggested that a  $\beta$  protodelta duplication occurred in a tarsoid-like line ancestral to both *Tarsius* and Anthropoidea<sup>27</sup>.

The  $\delta$  chains in New World monkeys, apes and man all have Arg and Asn in positions 116 and 117 respectively instead of His at both these sites which occurs in the  $\beta$  chains. Gly, however, is present at position 5 in both chains of the New World monkeys (except for *Cebus* which was Ala in the  $\beta$  chain) whereas Pro is present in both chains of apes and man. These latter data suggest that more than one gene duplication has occurred<sup>21,28</sup>.

Starch gel electrophoresis (in 6 M urea) indicated that the tarsier haemoglobins differed in their non- $\alpha$  chains. The amino acid compositions of those tryptic peptides containing the sites important in deciphering the origin of the  $\delta$  chains of primates (positions 116, 117 and 5) were identical in both haemoglobins of tarsier. There is, therefore, no direct evidence that the  $\beta$ -like chain of the minor haemoglobin of *Tarsius* is orthologous with either man-ape or New World monkey  $\delta$  chain. It should also be noted that the 'minor' component of tarsier is present in relatively high concentration (18% of total). In other species the concentration of minor component is between 0.5% and 6% (ref 21). It would be interesting to examine the haemolysates of other tarsier blood samples and look at the frequency of occurrence of a minor component in all species of tarsier. Two major haemoglobins have been observed in several prosimians<sup>8,29</sup>.

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## Thymine hydroperoxide as a mediator in ionising radiation mutagenesis

We describe the mutagenic action of chemically synthesised *cis*-5,6-dihydroxy-6-hydroperoxy-5-hydroxythymine (6-TOOH)<sup>1</sup> on transforming DNA of *Haemophilus influenzae*<sup>2,3</sup>, and suggest a mechanism by which ionising radiation induces mutations *in vivo*.

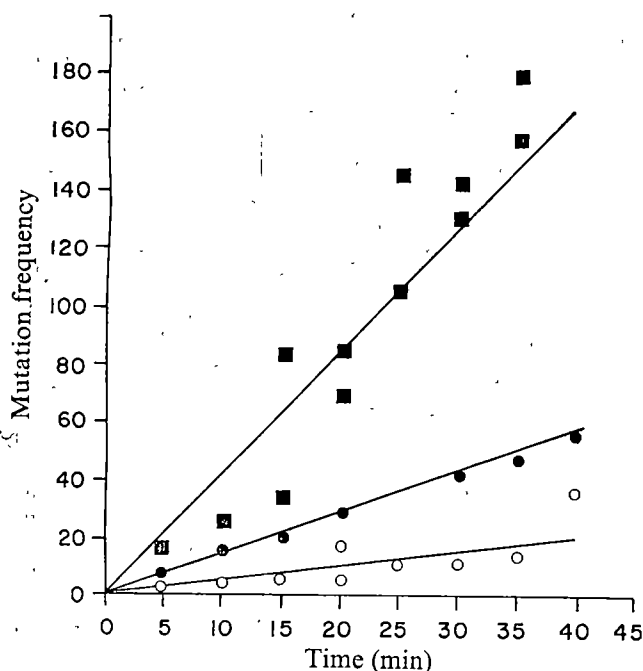


Fig. 1 Effect of 6-TOOH concentration on the induction of mutations in DNA. Weighed quantities of synthetic 6-TOOH<sup>1</sup> were mixed with solutions of *H. influenzae* DNA (10 µg ml<sup>-1</sup>) carrying the marker high resistance to streptomycin (2,000 µg ml<sup>-1</sup>). The solvent was 0.08 M Tris buffer, pH 7.8-0.04 M NaCl and 0.25 mM CuSO<sub>4</sub>. Samples from the reaction mixture, held at 25 °C, were removed at intervals diluted and assayed with competent *H. influenzae*<sup>3</sup> for markers carrying resistance of 8 µg ml<sup>-1</sup> of kanamycin and for the surviving streptomycin resistance. The mutation frequency is the ratio of the fraction of mutants (*M*) to the fraction of implicit marker (*S*); (*M*<sub>t</sub>/*M*<sub>0</sub>)/(*S*<sub>t</sub>/*S*<sub>0</sub>) where the values at time *t* are compared with that initially (0) present. The initial mutation frequency is unity and not zero as it might appear in the drawing. 6-TOOH concentration was: ○, 11.3 mM; ●, 22.6 mM; ■, 45 mM.

Since Muller<sup>4</sup> discovered radiation mutagenesis no molecular mechanism to explain it has been established. Irradiation of aqueous solutions of biological materials in the presence of air generates various peroxides, including hydrogen peroxide, pyrimidine hydroperoxides<sup>5-7</sup> and lipohydroperoxides<sup>8-10</sup>. Other organic peroxides<sup>11-15</sup> have been found to be mutagenic but they have not been identified in irradiated cells. Hydrogen peroxide is only weakly mutagenic for bacteria<sup>16</sup> and not at all for transforming DNA<sup>15</sup>. The mutagenic potential of lipohydroperoxides has been indicated but not established<sup>17</sup>. Thymine hydroperoxide was reported to be mutagenic for *Escherichia coli*<sup>18</sup>, but this did not get support<sup>19</sup> and later reviews<sup>20,21</sup> did not mention it. Our work began with the finding that 6-TOOH, the agent used by earlier workers<sup>18</sup>, was mutagenic for *H. influenzae*. But a mechanism of action is not easily traced in an *in vivo* system, so the relatively uncomplicated transforming DNA freed of other cellular components was chosen for study. Tris-buffered solutions of purified *H. influenzae* transforming DNA<sup>3</sup> were exposed to 6-TOOH in the presence of a transition metal ion. Changes in the transforming DNA<sup>3</sup> leading to mutations were recognised as new resistance to either of two antibiotics which the DNA conferred on competent *H. influenzae* that are normally sensitive to the antibiotics. Before treatment with hydroperoxide, the transforming

Table 1 Effect of metal ions on mutagenic action of 6-TOOH

Metal ion	Δ Mutation frequency per min
Cu <sup>2+</sup>	1.3
Co <sup>2+</sup>	0.35
Mn <sup>2+</sup>	0.1-0.3
Fe <sup>2+</sup>	0.2
Zn <sup>2+</sup>	0.2
None	0.08
Ca <sup>2+</sup>	0.08
Mg <sup>2+</sup>	0.08
Cu <sup>2+</sup> + 10 <sup>-3</sup> MEDTA	<0.02

The conditions of this comparison were those described in Fig. 1 except that the nature of the metal ion was varied. The initial concentrations of 6-TOOH was 22.6 mM and the metal ion was 0.25 mM in all instances.

DNA conferred resistance to streptomycin, a property which was followed to evaluate the destructive action of the hydroperoxide of genetic markers. Knowledge of this destructive action is necessary if the mutation frequency is to be calculated.

The mutagenic action of 6-TOOH in the presence of Cu<sup>2+</sup> is shown in the data plotted in Fig. 1. The frequency of colonies resistant to kanamycin increased linearly with time of exposure to 6-TOOH and the rate was roughly proportional to the hydroperoxide concentration. A comparable increase in frequency of resistance to novobiocin was also found. In very similar experiments, the effect of different transition metal ions on 6-TOOH mutagenesis was determined. The results are shown in Table 1. Clearly among the metal ions examined, Cu<sup>2+</sup> is much more effective than Co<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup> or Zn<sup>2+</sup> and two non-transition metal ions Ca<sup>2+</sup> and Mg<sup>2+</sup> which showed no mutagenic action. The level of metal ion represents that which was introduced and not the level of free ionic form during the reaction. The latter was almost certainly lower, for metal ions readily complex with constituents of the system to form weakly dissociated units. The dependence of 6-TOOH mutagenesis on the concentration of metal ion is seen in the results in Fig. 2. The rate of mutagenesis seems to be roughly a direct function of the Cu<sup>2+</sup> concentration. When EDTA was added, mutagenesis by the 6-TOOH was completely blocked. The weak, but definite, mutagenesis observed when no metal ion was added may

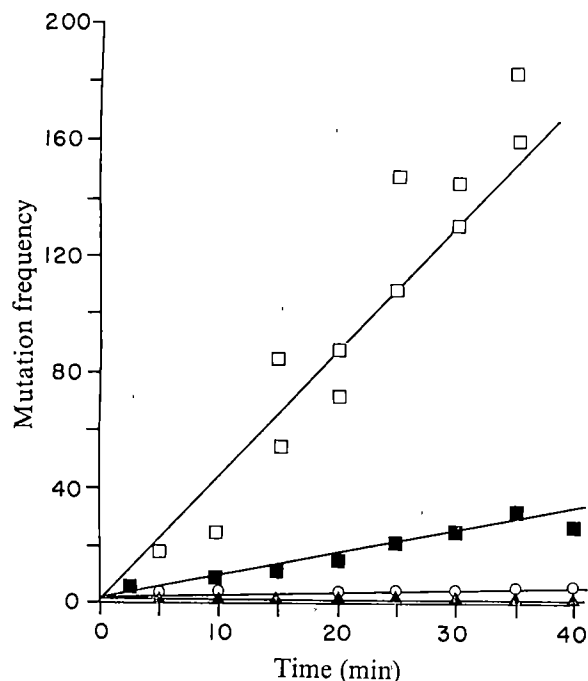


Fig. 2 Effect of copper ion concentration on the generation of mutations in DNA by 6-TOOH. The conditions were the same as described in Fig. 1 except that the 6-TOOH concentration was held at 45 mM and the  $\text{Cu}^{2+}$  was varied. ○, No added  $\text{Cu}^{2+}$ ; ■, 0.1 mM  $\text{Cu}^{2+}$ ; □, 0.25 mM  $\text{Cu}^{2+}$ ; ▲, 0.25 mM  $\text{Cu}^{2+}$  + 1 mM EDTA.

have been due to trace quantities of metal ions introduced with the DNA or the inorganic salts. Taken altogether, these results suggest that 6-TOOH in cooperation with certain metal ions, produces changes in transforming DNA that lead to mutations, although a detailed mechanism remains to be established. The involvement of transition metal ions and peroxides, which had been recognised earlier<sup>15,19</sup>, probably means that free radicals are involved<sup>22,23</sup>. Comparable mutagenic action of 6-TOOH on denatured and native transforming DNA seems to eliminate consideration of the denaturing effect of  $\text{Cu}^{2+}$  on DNA<sup>24,26</sup>. In the absence of metal ion, 6-TOOH produced changes in bases guanine, cytosine, and thymine<sup>27</sup>.

Pyrimidine hydroperoxides formed in DNA would be in close proximity to other bases and so might be highly mutagenic as a result of interaction, but neither these *in situ* hydroperoxides nor their glycol products of reaction would be planar and probably could not pair with other bases<sup>28</sup>. Excision of the pyrimidine hydroperoxides from DNA<sup>29,30</sup> *in vivo* would not be expected to reduce their mutagenic action below the level shown in the study reported here.

Hydrogen peroxide in the presence of  $\text{Cu}^{2+}$  failed to produce mutations in *Haemophilus* transforming DNA, which indicates that  $\text{H}_2\text{O}_2$  is not an active intermediate responsible for the mutagenic action of 6-TOOH. This was supported by the finding that catalase, which destroys  $\text{H}_2\text{O}_2$ , did not reduce the mutagenic power of 6-TOOH. Absolute increases in the mutant frequency in early samples of 6-TOOH mutagenesis of transforming DNA disposes of the argument that increases in the mutant frequency may be due to a selective advantage caused by a higher resistance of the mutants to the lethal action of the hydroperoxide. Studies (to be published) of the action of 6-TOOH on Ames' histidine mutants of *Salmonella typhimurium*<sup>31</sup> indicate that the type mutants produced by this agent are substitution rather than frameshift. In this respect, the action of 6-TOOH is similar to that of alkylating agents<sup>28</sup>.

Based on our results, the following mechanism for *in vivo* radiation mutagenesis is proposed, as a working hypothesis. Ionising radiation produces hydroperoxides of pyrimidines or their derivatives in the DNA or metabolic pool of cells. These hydroperoxides interact with transition metal ions forming free radicals which react with and modify neighbouring or other bases of the cell's genome. Some of these modified bases cause base mispairing during replication leading to what are generally regarded as mutations.

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# matters arising

## Clocklike behaviour of biological clocks

In a recent review article<sup>1</sup>, Winfree discussed a number of mechanisms that have been proposed to explain the dynamical nature of circadian clocks. The statement, "limit cycle excluded experimentally", appears as the heading of a section which proceeds to propose alternative mechanisms. Although the statements in the text qualify the experimental conclusions as excluding only a certain type of limit cycle, the rather sweeping statement in the heading is the one which is more likely to impress the mathematically unsophisticated reader. For this reason, we would like to emphasise some of the alternative mechanisms.

The data on which this argument is based result from two-pulse experiments<sup>2</sup>. After a relatively weak light pulse which perturbs the system, a second, much stronger one is applied. Because the *Drosophila pseudoobscura* eclosion rhythm can be monitored only for ten cycles or less, the second pulse follows the first by no more than 48 h: two cycles. It is found that the closer the first pulse brings the system to the hypothesised singularity, the flatter is the resulting, second-pulse resetting curve. The important point is that this flattening could be explained by a contraction in the net amplitude displayed during the two recovery cycles.

Winfree suggests two hypotheses to explain such a reduction. (1) A limit cycle process is not operative; rather, a single master oscillator displays conservative dynamics thereby allowing permanent amplitude reduction. (2) A limit cycle process is operative in a population of essentially uncoupled oscillators whose combined action gates the observed rhythm. Dephasing within the population will reduce the net amplitude displayed. Our aim is to suggest that other possibilities exist between these two extremes.

With regard to the first, Winfree has shown that recovery to the limit cycle most probably does not occur within two cycles. He proceeds, however, as though recovery never occurs, to exclude the possibility of a limit cycle model. It is easy to demonstrate that the two-pulse experiment data are compatible with a slowly recovering model. Figure 1 shows a phase plane plot

produced by computer simulation of one of the earlier models<sup>3,4</sup>. This particular plot shows the trajectory of a system starting very near the singularity. Strong pulses given to the system within the first two recovery cycles would result in flatter resetting curves the closer the initial pulse approaches the singularity. (Roughly speaking, the range of the resetting curve corresponds to the projection of a "cycle" on the  $S$  axis.) Similar responses can result from any system satisfying a set of rather general conditions<sup>5</sup>.

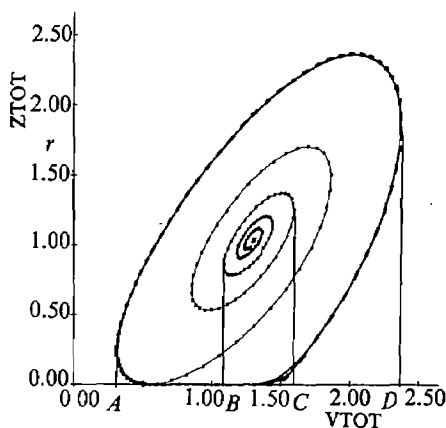


Fig. 1 CALCOMP plot of a trajectory starting near the singularity for the system described by differential equations:

$$\begin{aligned} dr/dt &= r - 0.8s - 0.3s^2 + 0.5 & r \geq 0 \\ ds/dt &= r - 0.8s \end{aligned}$$

This has been proposed as a model for circadian clocks<sup>3,4</sup>. Strong light pulses move  $r$  to zero so that the resetting zone, when the system is on the limit cycle, is  $AD$ . Two days away from the postulated initial condition, not far from the singularity, will be small,  $BC$ .

The gradual recovery from the singularity to the limit cycle is actually a property shared by many of the proposed models of circadian rhythms as well as other bio-oscillators<sup>5-8</sup>. Higgins has published a number of phase plots similar to our Fig. 1 (ref. 8). Wever has also described similar phenomena<sup>9</sup>. Quick recovery (that is recovery within a day) to the limit cycle has been the feature of some of the earliest models only and it was the result of an

attempt to simulate the effects of strong pulses.

Many other interesting possibilities occur if one uses models involving populations of coupled oscillators. Such models are quite popular<sup>4,9-11</sup> and have been successful in simulating phenomena such as frequency doubling (rhythm splitting)<sup>4,9-11</sup>. In a population of strongly coupled oscillators, each of which in isolation quickly recovers its limit cycle, a return to the original trajectory may be slowed by resynchronisation among the various units. Consider in addition, a population of units with a stable limit cycle surrounding both an antilimit cycle and stable singularity. If the units are not exactly identical, they will form a "cloud" along the limit cycle. Then the first pulse may send some units into the singularity's region of attraction while leaving others in the region of attraction of the limit cycle. Coupling between units would prohibit a quick return to the respective limit sets (cycle or singularity). Thus an intermediate trajectory will be followed, possibly with a split in the population in which one group resides near the singularity, the other near the limit cycle. A second strong pulse would drive both groups towards the limit cycle where mutual synchronisation would reform the original cloud. The closer the initial push to the singularity, the more units approach that point, and, in the limit, all units reside there indefinitely. In that case, a perfectly flat resetting curve results after the second pulse. (Amplitude was abolished.)

The number of models simulating two-pulse experiment results is certainly quite large and the choice among them may have to be made on the basis of other factors. We prefer limit cycle models (possibly involving strongly coupled oscillators) because they seem compatible not only with the data described in the literature on circadian rhythms<sup>4,11</sup> (including the two-pulse experiments), but also with other systems such as the metabolic oscillations in yeast, where behaviour similar to that of *D. pseudoobscura* is observed<sup>12,13</sup>. The yeasts are known to mutually synchronise within a cycle<sup>14</sup> and apparently are governed by limit cycle dynamics<sup>5,15</sup>. Furthermore, dynamical systems displaying conservative oscillations are structurally un-



stable in the extreme<sup>16</sup> and isoperiodic only in the most unusual conditions<sup>4,17</sup>.

In conclusion, we would like to emphasise that Winfree's elegant experiments have revealed certain aspects of the dynamic behaviour of the circadian regulator of the *Drosophila* eclosion rhythm and represent a prime example of the use of mathematical models for understanding the nature of biological mechanisms. It is only unfortunate that the paper chose to emphasise especially in its title and section titles only two of the many hypotheses still available. Furthermore, most of these hypotheses are very "clocklike" as long as the term "clock" is used to mean a general time-keeping mechanism.

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WINFREE REPLIES—There seem to be three issues, all resolvable. First, Aldridge and Pavlidis<sup>4</sup> rightly observe that my experiments<sup>2</sup> exclude only "quickly recovering" limit cycle models, by virtue of showing no change in a measure of the amplitude of a circadian oscillator during 48 h after each of 30 different adjustments of that amplitude. "No change" means roughly "less than  $\pm 10\%$ ". They rightly object that my adjective "quickly recovering", used everywhere else, was deleted from a section heading which then read "limit cycle excluded experimentally"—whereas a very slow recovery of amplitude remains unexcluded, as does a very slow decay of amplitude.

On the other hand, according to my data, the spiralling of trajectories in

the descriptive metaphor of their Fig. 1 is definitely too fast: almost all trajectories strike arc AD within 48 h, ensuring complete recovery to a standard cycle, contrary to observations.

Second, Aldridge and Pavlidis agree that my experiments suggest a long-lasting heterogeneity of state among two-to-many similar circadian mechanisms with each animal. I chose to emphasise phase incoherence and that this could derive from relative independence of these autonomous (possibly cellular) mechanisms. Assuming such independence and persistent incoherence in a population, the metaphor of Fig. 1 (among others) is perfectly compatible with the emphasised peculiarities of the circadian rhythm of *Drosophila*. This was intended to be the main point of my "Unclocklike . . ." paper in *Nature*<sup>4</sup>. Aldridge and Pavlidis chose to emphasise an alternative possibility, that some oscillators in the population are switched off, the others remaining synchronous. This model abandons Fig. 1 for a more complicated drawing with two limit cycles (one unstable) and supposes rapidly synchronising interactions among cells. I agree that this is a sensible alternative, which I overlooked. When a histological assay of circadian state becomes available, the two models—one predicting phase heterogeneity and independence, one predicting amplitude heterogeneity and coupling—should be clearly discriminable.

The third point is terminological. It is usual for familiar words, adopted into a specialised area, to change their connotation as perspectives change and scholars make more refined distinctions. As adopted in the 1950s "clock" connoted little more than adaptive stability of period. But by the mid-1960s much of the literature makes sense only if a more restrictive implicit connotation is recognised, namely what is distinguished in the cell-cycle literature<sup>4</sup> as a "simple clock". This is a mechanism which, like a commercial clock or a music box, unlike a dynamical oscillator, can change only its phase, having no states off a unique causal cycle. In pointing to the singularity and to amplitude lability as two "unclocklike" features of the circadian mechanism, I have adhered to this more restrictive usage, distinguishing "clocks" from "dynamical oscillators". In contrast, Aldridge and Pavlidis apparently use "clock" to mean "dynamical oscillator" as opposed to such alternative periodic mechanisms as a sequential state machine with one fixed cycle. Thus the "clocklike" features of their paper<sup>4</sup> are the same as the "unclocklike" features of mine<sup>3</sup>! I notice that Pittendrigh has simply abandoned the word in entitling a

current preprint ". . . circadian pacemakers".

I am delighted to have this critical exchange, and wish there were a lot more of it in the circadian literature.

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## Structure of the galactic magnetic field

RECENTLY Somogyi<sup>1</sup> has presented arguments indicating that the power spectrum of the turbulent galactic magnetic field is of the form  $k^{-\alpha}$ , where  $k$  is the wave-number and  $\alpha = 1.0-1.8$  depending on the model of cosmic-ray transport assumed. This spectrum is relevant for scales of 0.001-1 pc, and was obtained by noting the dependence of the cosmic-ray anisotropy and pathlength on energy. I wish to show that a similar conclusion follows from a related but independent argument based solely on the average cosmic-ray pathlength and observed properties of the large scale galactic magnetic field<sup>2</sup>.

Consider a simple one-dimensional model for the diffusion of cosmic rays perpendicular to the plane of the Galaxy, with constant diffusion coefficient  $K_1$  and free escape at the boundaries  $z = +L$  and  $z = -L$ . For observers near the galactic disk ( $z = 0$ ) it is straightforward to show that the mean path  $\lambda$  is given by

$$\lambda = nm_{\text{HC}}\langle t \rangle = (5/12)nm_{\text{HC}}L^2/K_1 \quad (1)$$

where  $n$  is the interstellar density of hydrogen,  $m_{\text{H}}$  is the mass of a hydrogen atom,  $c$  is the speed of light, and  $\langle t \rangle$  is the average cosmic-ray lifetime<sup>2,3</sup>. From equation (1), one may infer a value of  $K_1$ , given the other parameters, and for those listed in Table 1, the value of  $K_1$  varies from  $1.2 \times 10^{26}$ - $1.2 \times 10^{28}$  cm<sup>2</sup> s<sup>-1</sup>, with a nominal value of  $\sim 10^{27}$  cm<sup>2</sup> s<sup>-1</sup>. This value corresponds to the 'observed' value of the cosmic-ray diffusion coefficient for those particles (with energies  $\sim 5$  GeV per nucleon) which are responsible for spallation.

Next consider the derivation of the diffusion coefficient from the properties of the random interstellar magnetic field. Using standard diffusion theory<sup>4,5</sup>, in the limit that the gyroradius  $r_g$  of the particle is much smaller than the magnetic-field correlation length  $l_0$ , one obtains<sup>2,6</sup>

$$K_1 = F(\alpha)cl_0(r_g/l_0)^{2-\alpha} \quad (2)$$

Here  $F(\alpha)$  is a factor of order unity<sup>2</sup>, and it has been assumed that the magnetic-field power spectrum is flat for wave

numbers  $k < (l_0)^{-1}$ , and is proportional to  $k^{-\alpha}$  for larger wave numbers. Jokipii and Lerche<sup>7</sup> give  $l_0 = 150$  pc, and find an average interstellar field of  $3 \times 10^{-6}$  gauss, so for  $\sim 5$  GeV per nucleon particles,  $r_g/l_0 \sim 1.4 \times 10^{-8}$ . Because this ratio is so small,  $K_1$  as determined from equation

Table 1 Adopted parameters

Parameter	Nominal value	Range
$n$	$1 \text{ cm}^{-3}$	0.2–2
$L$	$1.5 \times 10^3 \text{ pc}$	1.0–3.0
$\lambda$	$5 \text{ g cm}^{-2}$	3–8
$l_0$	$1.5 \times 10^2 \text{ pc}$	0.5–2.0
$B_0$	$3 \times 10^{-6} \text{ gauss}$	2–5

(2) is very sensitive to the exponent  $\alpha$  of the magnetic-field power spectrum. The values calculated from equation (2) are given in Table 2 for several values of  $\alpha$ . The uncertainties given in Table 2 are obtained by varying the parameters through the range shown in Table 1. Comparing the 'observed' result from equation (1) with the entries in the table, one concludes that

$$\alpha = 1.5 \pm 0.2 \quad (3)$$

if the simple diffusive model of cosmic-ray transport is appropriate. The uncertainty quoted in equation (3) is the worst-case limit; the statistical uncertainty would be much less.

This value of the power spectral index ( $\alpha \sim 1.5$ ) of the galactic magnetic

Table 2 Calculated diffusion coefficients

$\alpha$	$K_1 (\text{cm}^2 \text{ s}^{-1})$
1.2	$(5.3 \pm 3.0) \times 10^{24}$
1.4	$(1.5 \pm 0.8) \times 10^{26}$
1.5	$(1.0 \pm 0.5) \times 10^{27}$
1.6	$(6.9 \pm 4.0) \times 10^{27}$
1.8	$(4.6 \pm 3.0) \times 10^{29}$

field is the same as the exponent of the interplanetary field and close to the Kolmogorov value of 5/3. The argument here includes the assumption of one continuous power law from the correlation scale of the fluctuations (150 pc) all the way down to the resonant wavelength for 5 GeV per nucleon particles ( $2 \times 10^{-8}$  pc). For comparison, Somogyi obtains  $\alpha = 1.7 \pm 0.1$  in the simple diffusion model for cosmic-ray transport with the distance to the boundary of the diffusing region independent of energy. His result is based on the resonant wavelengths for  $10^{12}$ – $10^{15}$  GeV per nucleon particles, or scales of  $4 \times 10^{-4}$ – $0.4$  pc.

Somogyi's result and that of equation (3) can be shown to agree even more

closely if one adopts his model and changes his parameters slightly. If the energy dependence of the cosmic-ray pathlength  $\lambda(E) \sim E^{-\beta}$ , with  $\beta = 0.50 \pm 0.1$ , as recent calculations indicate<sup>8</sup>, rather than  $\beta = 0.2 \pm 0.1$  as adopted by Somogyi, then his results indicate that the size of the confinement volume does not depend on energy, and that  $\alpha = 1.55 \pm 0.1$ , in agreement with equation (3). Since equations (1) to (3) yield  $\beta = 2 - \alpha$ , the calculations in this letter predict  $\beta \sim 0.5$ , in agreement with the conclusions of Juliusson *et al.*<sup>8</sup>.

Thus the method presented here gives the result  $\alpha = 1.5 \pm 0.2$ , and predicts that the cosmic-ray pathlength varies as  $E^{-1}$ . Adopting this dependence of pathlength on energy, as supported by recent observations, one can use Somogyi's results to obtain  $\alpha = 1.55 \pm 0.1$  and to show that the size of the diffusing region is independent of energy. The similarity of these two results, based on quite different assumptions, suggests that cosmic rays may be a useful tool for probing the interstellar magnetic field.

I thank Dr W. R. Webber for calling my attention to Somogyi's paper, and for his hospitality while this letter was being written. This work was supported by the Research Corporation through a Cottrell College Science Grant.

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**SOMOGYI REPLIES**—The value of  $\alpha$  as derived by Owens<sup>1</sup> is based on a single point of the function expressed by his equation (2), and Owens assumed that the magnetic power spectrum had the form of a power function with a constant exponent in the range  $2 \times 10^{-8} < k^{-1} < 150$  pc. In my paper<sup>2</sup>, indications are given that the anisotropy and lifetime are power functions of energy, and it is proved that in this case the magnetic power spectrum is a power function at least in the range  $10^{-3} \text{ pc} < k^{-1} < 1 \text{ pc}$  corresponding to the energy range  $10^{12} \text{ eV} < E < 10^{15} \text{ eV}$  of anisotropy measurements with large statistical accuracies. The range of  $k$  as given in my paper<sup>2</sup> is thus an experimentally established one.

It would be difficult to argue which of the two  $\alpha$  values is better established. Both are rather uncertain. I agree with

Owens that their agreement is remarkable, especially if recognising that they are based on different experimental evidences independent of each other.

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- <sup>1</sup> Owens, A. J., *Nature*, **259**, 344–345 (1976).
- <sup>2</sup> Somogyi, A. J., *Nature*, **225**, 689–690 (1975).

## How specific are nuclear 'receptors' for thyroid hormones?

TATA has questioned<sup>1</sup> the biological relevance of the binding of  $T_3$  to the nucleus. He presents four main arguments against a physiological role for the  $T_3$  binding components in the nucleus. First, the presence of high-affinity, saturable binding sites for thyroid hormones of similar characteristics in a number of other subcellular fractions. Second, the lack of analogy with steroid hormone receptors. Third, the absence of parallelism between the binding of thyroid hormone analogues to the nucleus and the biological activity. Fourth, the fact that only 15–20% of the intracellular triiodothyronine is located in the nucleus.

We believe that several of Tata's conclusions are invalid.

On the first point. The  $K_a$  of the binding of  $T_3$  by the nucleus has been incorrectly cited from the work of Samuels and Tsai<sup>2</sup> (Tata's ref. 19). The correct value is  $3.3 \times 10^{10} \text{ mol}^{-1}$  ( $K_d = 29 \text{ pmol}$ ) for the nuclei of GH<sub>1</sub> cells. The same high  $K_a$  has been found<sup>3</sup> for the binding of  $T_3$  to the nucleus of human lymphocytes and rat liver and kidney nuclei<sup>4</sup>. The fact that these values are much higher than those reported by Tata for the other subcellular components favours the possibility of a physiological role for this type of binding site within the nucleus.

With the technique used by Tata to measure specific binding (his Fig. 2) it is impossible to show high affinity binding sites in the nucleus, because the tracer concentration used is 60 times the maximal binding capacity in the incubation mixtures (binding capacity  $2.9 \text{ fmol } T_3 \text{ per } 100 \mu\text{g DNA}$ ; see ref. 4).

Concerning the second point, the lack of analogy with steroid hormone receptor binding has been reported by Surks *et al.*<sup>5</sup> (Tata's ref. 17) and Visser *et al.*<sup>6</sup>. Why does this lack of analogy imply that thyroid hormone binding in the nucleus is without physiological relevance? Why should the nuclear binding of chemically different substances proceed along identical lines?

The *in vitro* binding of  $T_3$  to the nucleus diminishes when cytosol is added, because the cytosol competes for the same fixed amount of hormone in the incubation mixtures<sup>4</sup>. But, *in vivo* that is not the case since both cytosol binding proteins and nuclear binding sites are in equilibrium with the same free hormone concentration outside the cell. This implies that the presence of more or less binding sites outside the nucleus probably has no effect on the degree of saturation of the nuclear binding sites with  $T_3$ .

As for the third point, the parallelism between the nuclear binding of thyroid hormone analogues and hormonal activity has been shown by Oppenheimer *et al.*<sup>6</sup> (not cited by Tata). Only Triac may be an exception. The completely different manners of binding of thyroid hormone analogues with cytosol and the nucleus<sup>4,7,8</sup>, which implies a non-parallelism with biological activity, also suggests a physiological role for the  $T_3$  binding components of the nucleus.

Finally Tata's fourth point presents no argument at all. For instance, does the fact that more than 60% of thyroxine in the human body is outside the cells imply that this compound has no intracellular action? On the contrary, it implies that the cell has a constant supply of the hormone, independent of variations in production. Perhaps a large binding capacity of  $T_3$  outside the nucleus serves a similar purpose in the supply of  $T_3$  to the nucleus.

We think that the arguments used by Tata are insufficient to reject the postulate that the binding of thyroid hormones to the nucleus is of physiological importance.

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- <sup>1</sup> Tata, J. R., *Nature*, **257**, 18–23 (1975).
- <sup>2</sup> Samuels, H. H., and Tsai, J. S., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3488–3492 (1973).
- <sup>3</sup> Tsai, J. S., and Samuels, H. H., *J. clin. Endocr. Metab.*, **38**, 919–922 (1974).
- <sup>4</sup> Visser, T. J., Docter, R., Stunis, J. T., Bernard, B., and Hennemann, G., *Thyroid Hormone Metabolism* (edit. by Harland, W. A., and Orr, J. S.), 35–45 (Academic, London 1975).
- <sup>5</sup> Surks, M. I., Koerner, D., and Oppenheimer, J. H., *J. clin. Invest.*, **55**, 50–60 (1975).
- <sup>6</sup> Oppenheimer, J. H., Schwartz, H. L., Dillman, W., and Surks, M. I., *Biochem. biophys. Res. Commun.*, **55**, 544–550 (1973).
- <sup>7</sup> Dillman, W., Surks, M. I., and Oppenheimer, J. H., *Endocrinology*, **95**, 492–498 (1974).
- <sup>8</sup> Koerner, D., Surks, M. I., and Oppenheimer, J. H., *J. clin. Endocr. Metab.*, **38**, 706–709 (1974).
- <sup>9</sup> MacLeod, K. M., and Baxter, J. D., *Biochem. biophys. Res. Commun.*, **62**, 577–583 (1975).
- <sup>10</sup> Charles, M. A., Ryffel, G. U., Obinata, M., McCarthy, B. J., and Baxter, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1787–1791 (1975).

TATA REPLIES—I apologise for the errors (see corrigendum, this page) in

my original article<sup>1</sup>. Nonetheless they do not in any way modify the essence of my arguments and conclusions, which Docter *et al.*<sup>2</sup> have misread.

The absolute value of  $K_a$  for the interaction between the isolated nucleus and thyroid hormone is determined by the experimental conditions and is not relevant to my finding that in identical conditions all the subcellular fractions of rat liver exhibit rather similar  $T_3$ -binding constants and properties. Unless similar experiments comparing nuclei with extranuclear fractions are performed by others, I do not see how  $K_a$  values of the order of  $3 \times 10^{10} \text{ mol}^{-1}$  for isolated nuclei establish 'specificity'. Sterling and Milch have observed<sup>3</sup>  $K_a$  values of  $10^{11} \text{ mol}^{-1}$  for the binding of  $T_3$  to mitochondrial extracts. Unpublished data from my own laboratory reveal that concentrations of  $T_3$  lower than those reported in my article did not appreciably alter the result I reported there<sup>1</sup>. A study based on electron microscope autoradiography has also disclosed an ubiquitous intracellular distribution of thyroxine<sup>4</sup>. I agree with Docter *et al.*

## Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

that it is important to consider the situation *in vivo*, but their reasoning (their second point) can equally well be reversed: that is, the number of  $T_3$ -binding sites within the nucleus may have little effect *in vivo* on the saturation of extranuclear sites.

As regards the parallelism between the relative binding affinities and physiological potencies of different hormone analogues (their third point), it would be desirable to observe it in any binding or receptor system. But such an analogy *per se* is of limited value since it is also observed in binding to inert materials like glass, paper, talc, and so on<sup>5,6</sup>. All of these con-

siderations only highlight the difficulties of extrapolating from the results of interaction between isolated subcellular preparations and hormone 'receptors' *in vivo* of physiological significance.

Finally, as perhaps the first person to have pointed out the importance of the response of the target cell's nucleus to thyroid hormones, I would not like to rule out the possible location of hormone 'receptors' in that organelle. What I have, in fact, emphasised in my article<sup>7</sup> is the necessity for a more critical assessment of the current approaches based on the binding of thyroid hormones to isolated nuclei. As, and when, evidence is presented that such binding is related to a primary biochemical event leading to the physiological action of the hormone, then I shall be only too pleased to acknowledge that I am wrong in suggesting this note of caution and necessity for fresh thinking. Meanwhile, the past 50 years' history of thyroid hormone action has taught me not to be too dogmatic or 'fashionable'.

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- <sup>1</sup> Tata, J. R., *Nature*, **257**, 18–23 (1975).
- <sup>2</sup> Docter, R., Visser, T. J., and Hennemann, G., *Nature*, **259**, 345–346 (1976).
- <sup>3</sup> Sterling, K., and Milch, P. O., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3225–3229 (1975).
- <sup>4</sup> Manuelidis, L., *Yale J. biol. Med.*, **45**, 501–518 (1972).
- <sup>5</sup> Tata, J. R., *Rec. Progr. Horm. Res.*, **18**, 221–259 (1962).
- <sup>6</sup> Cuatrecasas, P., and Hollenberg, M. D., *Biochem. biophys. Res. Commun.*, **62**, 31–41 (1975).

## Corrigendum

In the article "How specific are nuclear 'receptors' for thyroid hormones" by J. R. Tata (*Nature*, **257**, 18; 1975) the following corrections should be made.

On page 21, lines 22–26, the sentence should read . . . This is in agreement with recent reports from Baxter's laboratory<sup>23,40</sup>, but whether or not the endogenous hormone-receptor complex is directly bound to DNA as concluded by these workers or merely to non-histone protein in intact chromatin, as proposed by others<sup>14,15,20–22</sup>, is difficult to decide . . .

In Table 3 (page 22), the following corrections should be made:

The  $K_a$  ( $M^{-1}$ ) value for rat pituitary tumour cell line nuclei should read  $3.3 \times 10^{10}$ .

The no. of sites for rat liver and kidney cytosol should read 0.53 pmol  $mg^{-1}$  liver and 2.9 pmol  $mg^{-1}$  kidney, respectively.

References 28,29,30 should read 29,30,31.

# reviews

*The Evolution of IBP.* (International Biological Programme 1.) Edited by E. B. Worthington. Pp. xx+268. (Cambridge University: Cambridge, London, New York and Melbourne, September, 1975.) £10.50. *Food Protein Sources.* (International Biological Programme 4.) Edited by N. W. Pirie. Pp. xx+260. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £7.50.

IN March 1959, shortly after the International Geophysical year ended, Sir Rudolph Peters conceived the idea of its biological equivalent. The idea gained the support of a number of biologists, although each had a different idea of what should be studied. In this way the International Biological Programme (IBP) was born. Through the efforts of the late Professor C. H. Waddington and Professor Guiseppe Montalenti, it had by 1964 grown into a decade long, seven-sided study of production ecology and human biology, entitled "The biological basis of productivity and human welfare." Now, nearly 20 years after its conception, the results are to be gathered in an 'international synthesis', a series of volumes of which Dr Worthington's kaliedoscopic study *The Evolution of IBP* is the first.

Dr Worthington was the scientific director of IBP, and the grasp of all its aspects which he demonstrates in this book, shows why Professor Waddington was so determined he should be appointed to that post. He modestly describes himself as editor of the book, although he is virtually its author and, as such, does an extremely workmanlike job of describing what IBP was. But for all its assiduous and dispassionate detail, Dr Worthington's book is not a history of IBP but a historical gazeteer, circumscribed by its own objectivity.

I suspect this has arisen paradoxically from Dr Worthington's deep involvement with the programme, for those so caught up in events are often the worst historians. Though Dr Worthington avoided producing an apologia for IBP he has not produced a critique either. He has simply chosen to record, rather than interpret, events. It is not that he sees history from the IBP's viewpoint: apart from IBP, he does not seem to see history at all. And so the events that have altered scientists' attitudes while IBP existed—events that must have moulded and been

moulded by IBP—are avoided, or only mentioned coyly. Dr Worthington's book is interesting and readable but IBP still needs its Margaret Gowing.

As does the topic to which Mr Pirie has addressed himself for the past 40 years—the need for increasing world protein production. Mr Pirie has long been the advocate of leaf protein as one method of bridging the world protein 'gap' and he persuaded IBP to stress leaf protein in one of its themes.

## International biological programme

Unfortunately, over the years the protein gap has not so much been bridged as obliterated by systematic reductions in estimates of human protein requirements. Mr Pirie has not appreciated this alteration. Discussing the matter in 1969 he commented on the technical competence and professional integrity of some of those responsible for these estimates suggesting that they were biased by a desire to avoid change and a wish to resist wage demands based on the cost of food.

In this volume he avoids those issues and states simply that "more research is needed to meet the world's.....protein need than.....the equally important need for energy and vitamins". I do not believe it and suspect that Mr Pirie underestimates the inertia of his own ideas.

The definition of a protein source used in this book (at least 15% protein on a dry weight basis) is unfortunate, since it excludes the cereals, which provide over half the protein in most diets. Further complications are introduced by the exclusion of domesticated livestock and the inclusion of coconuts and minor seeds which contain less than 15% protein, but which are "interesting and liable to be overlooked".

The discussion of nutritive value is concentrated on protein value, and is confused by the use of terms like BV, NPU and PER without definition or details of the assay conditions. It is a pity that the IBP committee on units symbols and definitions which produced

the 50 page appendix to Dr Worthington's book omitted these. One suspects that some authors did not even consult IBP's own symposium on the evaluation of protein products.

Parts of the book are good—Sir Kenneth Blaxter on non-domesticated ruminants, for example—but on the whole it is a patchy compendium of some information on some aspects of some foods.

The power of the protein myth is such that its title assures it of a good sale, but I wish that Mr Pirie had devoted his talents and his time to a book on food and not fashion.

Fashion is perhaps the nub of it. It is inconceivable that an IBP started now would give such a high priority to protein. Nor that it would so easily dismiss Margaret Mead's plea for basing its Human Adaptability Programme on the social sciences. But then would a new IBP have such difficulty in getting a programme of production ecology started and funded?

IBP was overtaken by events, as anything started in 1959 had to be. In 1959 there was Eisenhower and Khrushchev. No one had heard of Yuri Gagarin, and very few of James Watson. Ecology was still about beetles.

IBP's operational decade encompassed Biafra and Vietnam. At its end there were famines in the Sahel and Ethiopia. A study of the biological bases of human welfare conceived now would be very different indeed: IBP seems academic, old fashioned and even slightly naïve.

Some of its synthesis volumes—the academic ones—will no doubt be standard texts, as some of its achievements are undeniable. Those like Mr Pirie's that are concerned so directly with human welfare run the risk of being outdated by the time they appear. Whether in the long run they'll be seen as the more significant or noble, remains to be seen.

John Rivers

*Crop Genetic Resources for Today and Tomorrow.* International Biological Programme 2.) Edited by O. H. Frankel and J. G. Hawkes. Pp. xiv+491. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £13.

IN recent years there has been an increasing awareness of the erosion of plant genetic resources due to the extension and intensification of land



use required to meet the demands of the human population for food and for manufactures. The concern that action should be taken to conserve these non-renewable resources has been displayed by a relatively few committed workers, among whom the editors of this book—Sir Otto Frankel and Professor Jack Hawkes—have been prominent. The institutional acceptance of the need for co-ordinated action first resulted in a joint review of the problem by the UN Food and Agriculture Organisation (FAO) and IBP. The outcome of this was the publication, as an IBP Handbook, of *Genetic Resources in Plants—their Exploration and Conservation* (edit. by O. M. Frankel and E. Bennett, Blackwell, 1970).

FAO continued to be active in the field or plant exploration and conservation through an Expert Panel. This led in 1974 to the establishment of an International Board for Plant Genetic Resources (IBPGR) under the auspices of the Consultative Group on International Agricultural Research which, co-ordinated by the World Bank, channels funds from governmental and non-governmental donors into the international research centres. The involvement of the IBPGR in plant exploration and conservation marks the initiation of a new phase in the development of this work and one in which it may be expected that wide scale effort will be backed by adequate resources.

Whatever happens in the new phase, however, it will be necessary for those concerned to draw to a large extent on the philosophies and methodologies developed during earlier work. These are well portrayed in *Crop Genetic Resources for Today and Tomorrow* which shows the point reached at the end both of the decade of the IBP and of joint work between FAO and IBP. The book contains contributions made at a technical conference organised by FAO and IBP, one of the important objectives of which was to produce a book collating a range of relevant subjects and approaches. It achieved this objective very well.

The subjects covered included the nature and basis of genetic variation in plant populations and how the explorer should sample populations and regions. Attention is paid to the ways in which, in crop plants, material collected from the wild or in primitive cultivars can be used to provide agriculturally valuable disease-, insect-, drought-, or frost-resistant parents in breeding programmes. As would be expected there is extensive discussion of the problems of how living plant material can be stored as seed, pollen, tissue or cells in culture, or as sporophytic plant members such as roots,

tubers or woody branches. Another approach to 'storage' is to protect plant populations *in situ*, and this is discussed in terms of population genetics by S. R. Jain. The storage of data relating to conserved plants, and their retrieval, is of crucial importance if the collections are to be used effectively by breeders, possible methods are discussed by D. J. Rogers and others.

This book is principally concerned with genetic conservation for plant-breeding purposes. The collectors and



Larger than life winged figure carrying a buck, probably Persian. Engraved by W. Holt from a relief in the Palace of Ashurnasirpal II at Nimrud. Taken from *Fallow Deer: Their History, Distribution and Biology* by D. and N. Chapman. Pp. 256. (Terence Dalton, Lavenham, Suffolk, UK, 1975.) £7.80. A whole chapter is devoted to Persian fallow deer, a very rare and endangered species.

the conservers are therefore undertaking a service for breeders. It would seem desirable on future occasions when the topic is discussed for consideration to be given to the methods by which genetic variability can be transferred in breeding programmes because this defines the systematic range that interests breeders and the kinds of genetic variation that may be useful.

There would have been increased stimulation in the book if the organisers of the conference had invited a devil's advocate to marshal the arguments against conservation instead of entirely confining themselves to the 'in group'. Good arguments can be advanced against the idea that we can always generate the required genotypes by mutation or by the release of variation following hybridisation but no opportunity was given for their expression. This would perhaps have caused

greater emphasis to be placed on the need to conserve not individual alleles but those complexes of genes that have become coadapted over long periods of selections. This was not ignored but was in my view not given the necessary weight.

I am also surprised that no attention was paid at the conference to the conservation of non-nuclear genes. We knew in 1970 that susceptibility to the 'T' race of *Helminthosporium* which caused serious maize losses in the US was determined cytoplasmically. The susceptibility is now known to be determined by mitochondrial DNA. There are important genes in both the mitochondria and the chloroplast DNA. This is well displayed by S. G. Wildman and his colleagues in relation to variation in the large subunit of Fraction 1 protein which is coded for in chloroplast DNA. In considering the kinds of resources we must conserve, and their selection and evaluation, it will be increasingly necessary in the future to give thought to organelles.

This book should provide a useful primer on crop plant collection, conservation and utilisation at a time when such is badly needed **Ralph Riley**

*Photosynthesis and Productivity in Different Environments.* (International Biological Programme 3.) Edited by J. P. Cooper Pp xxiv+715. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £22.00.

THE main aim of IBP was the international study of the biological basis of productivity and human welfare and it is natural, therefore, that much of the research effort which IBP engendered should have centred on the primary stage of energy conversion, photosynthesis. The informed management of the world's resources and the support of its human population is dependent on a knowledge of this process in which solar energy becomes available for the nutrition of animals and man. Surprisingly little data was available on the productive potential of different natural and seminatural vegetation types until the initiation of the IBP in 1964 and much of the research which has since been conducted has been concerned with the accumulation of such vital information. Participants in this exercise gathered together in Aberystwyth in 1973 to present summaries of their findings and this book is based on a selection of the papers which were read at that Conference.

Perhaps the emphasis should be placed on the summary aspect of this collection of papers. One thing which has resulted from the decade of IBP is the assembly of vast quantities of

measurements of plant growth, taken both in the laboratory and the field, and it is obvious that no single volume could attempt to cover this material in any detail. This book does, however, set the scene for the more detailed reports, which have either preceded it or may now be anticipated. It is therefore aimed at the general biologist and presents a glimpse of the full spectrum of IBP work, but at the same time the fact that many papers contain previously unpublished results will make it appeal to research workers in the immediate field of productivity.

The first two parts of the book (out of a total of seven) are concerned with data accumulated on the primary production of terrestrial and aquatic ecosystems. Some of the individual papers within these parts are very broad, for example about 35 pages each on the productivity of forests, grazing lands and tundra, respectively. Such summaries must have been very difficult to write but more effort to avoid a parochial approach could have been made, especially by Kira, whose paper on forests is illustrated almost entirely by examples from Japan. The paper on grazing lands by Caldwell pays particular attention to the relative importance of the C4 pathway of photosynthesis in these habitats and provides a useful synthesis of this subject.

Although about a third of the earth's surface is technically arid (potential evapotranspiration exceeding precipitation), this is not reflected in the volume of literature relating to these situations. The inclusion of a chapter on primary production in deserts is therefore particularly welcome, especially the section dealing with the contribution of lichens to the energy input of such ecosystems.

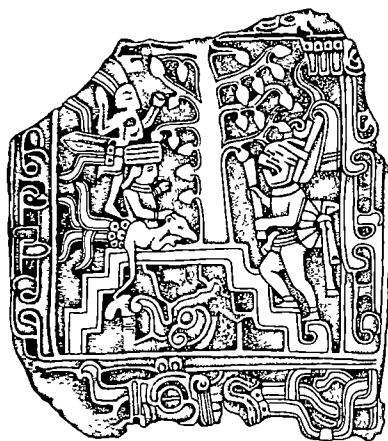
If the surface area of the earth occupied by an ecosystem is the grounds for justifying its inclusion in this volume, then the agricultural ecosystems of the world certainly deserve a place. A chapter by Loomis and Gerakis is given over to this subject. They concentrate on crop adaptations and show how the photosynthetic characteristics for which selection has been made in domesticated plants varies with latitude. C4 species being little used in latitudes higher than 40°.

Aquatic ecosystems, both freshwater and marine, are discussed at some length and the contribution to productivity by macrophytes and microphytes is considered. The figure derived for the global production of marine microphytes in these chapters ( $31 \times 10^9$  t carbon  $\text{yr}^{-1}$ ) is in close agreement with previously published figures.

The second half of the book turns from the study of whole ecosystems to more physiological aspects of the behaviour of light in plant canopies, photosynthetic studies of individual

species and the influence of environmental stresses upon photosynthesis and the use which is made of photosynthates.

Perhaps the most important contribution which this book makes is that it brings together reviews, most of which are extensive and well written, on a wide range of subjects from whole ecosystems, through whole plant physiology to subcellular biochemical ones. In doing this it may encourage workers in one field to become more aware of developments in related areas and to apply this knowledge to their own problems. Since the communication of ideas seems to be one of the greatest



Cocoa tree shown in bas relief on a Mayan stone tablet (13 × 1.3 cm) of the Late Classic Period (AD 600–900, from El Tajin, Veracruz, now at Instituto Nacional de Antropología e Historia Jalapa, Veracruz, Mexico. Taken from *Diseases of Cocoa* by C. A. Thorold. Pp. viii + 423. (Clarendon: Oxford, 1975) £11 00.

stumbling blocks to the general advancement of science, this book must be considered a valuable contribution. Indeed, if IBP had achieved no more than the bringing together of ecologists, physiologists and biochemists, then it would still have been worthwhile.

The price of this book precludes a very wide market, outside the better-endowed libraries, and this is unfortunate because many of the contributions would have been very useful to research workers and students alike.

**P. D. Moore**

*Small Mammals: Their Productivity and Population Dynamics.* (International Biological Programme 5.) Edited by F. B. Golley, K. Petrusewicz and L. Ryskowski. Pp. xxv + 451. (Cambridge University: Cambridge, London, New York and Melbourne, September 1975.) £12.

NINE years of collaboration by small mammal biologists from twenty-seven countries led to this volume, number 5

in the IBP series. No-one, least of all the editors and contributors, would claim that all there is to be known about small mammals has been fully researched and documented but this does not mean that the present work has had too short a gestation period. Its birth is timely, its development has been satisfactory and its information content is something on which to reflect. For the first time we are provided, within the covers of one book, with an overview of developments in the global study of small mammals. Theory, practice, results and hypothesis, as related to small mammal productivity and population dynamics, all have their place.

The contributors seem to have experienced difficulty in defining the upper size limits of a small mammal but 5 to 6 kg is among the highest figures quoted; bobcats (*Lynx rufus*) can therefore be considered as representing a large 'small mammal' (chapter 8). Among the small mammals, however, most information derives from rodents and it is not surprising that much of the text is devoted to this group of animals. Needless to say gaps in our knowledge of small mammals are referred to on both a taxonomic (For example, bats) and geographical (For example, the tropics) scale.

The ecological implications of being a small mammal are highlighted in the introduction and the importance of these animals as consumers within natural and man-modified ecosystems is clearly indicated. This theme is returned to later, particularly in chapters 10–13, in which the role of small mammals in arctic, temperate, tropical, and eurasian desert systems is reviewed. It is also referred to in chapter 14, in which control measures for small mammals which are either agricultural pests or disease vectors are stressed.

To study the status and role of a small mammal population within any ecosystem it is not only necessary to have a clear idea of the aims of the study but also some idea of the methods to be used in achieving the objective. The second part of the introduction provides a theoretical treatment of productivity investigations and as such provides a useful background to chapters 2–9. Early chapters cover aspects such as population density estimation and methods of age determination. Demographic patterns are reviewed on a worldwide scale and the role of dispersal in population regulation is discussed. Chapter 6 provides evidence of the value of morpho-physiological indices as predictors of the present and future state of population processes, particularly those concerned with population fluctuations. The three fol-

## Birth of a membrane

*Membrane Biogenesis: Mitochondria, Chloroplasts and Bacteria.* Edited by Alexander Tzagoloff. Pp. xvi+460. (Plenum: New York and London, 1975.) \$27.

THIS collection of articles attempts to review the present state of knowledge and direction of research concerning the mechanisms by which biological membranes are assembled. The various contributors are all expert in their field, and give accounts largely of their own work with only sufficient review material as might be necessary to provide perspective.

About 40% of the book is concerned with yeast or *Neurospora* mitochondria, in articles from the laboratories of H. R. Mahler, A. Goffeau, A. W. Linnane, H. W. Weiss and R. A. Butow. The level of investigation described ranges from the more molecular (Weiss on cytochromes) to the more biological, and of the latter class most are concerned with nuclear-cytoplasmic relationships rather than membrane synthesis *per se*. How far the more biological levels of research will proceed towards a deeper understanding of membrane biogenesis rather

than of mitochondrial genetics is not yet clear, and several of the articles would not be out of place in a volume that was dedicated to some subject quite different from membrane biogenesis. Nevertheless these excellent articles are representative of the field as it is and serve to illustrate the features of control and location that complicate the mechanistic aspects of membrane biogenesis in the mitochondria of eukaryotic cells. It is probably these very complexities which serve to divert workers on eukaryotic cells from some of the central problems of membrane biogenesis, well identified in Tzagoloff's introductory chapter. The same concern with nuclear-cytoplasmic relationships arises in the articles by L. Bogorad, R. J. Ellis and I. Ohad on the biogenesis of chloroplast membranes, which account for 33% of the book.

The remaining 27% of the book consists of articles by M. Inouye, L. Minditch and M. Bayer respectively on the outer, inner and outer-plus-inner cell membranes of *Escherichia coli*. The article by Inouye shows well the penetrating knowledge that can be acquired using molecular and biological approaches with *E. coli*: it could serve as compulsory reading for all who wish to work on membrane biogenesis, for it leads the way in setting up (and achieving) the sort of detailed mechanistic objectives necessary for an understanding of how cells assemble their membranes. Although the outer membrane of *E. coli* may not be very representative of more metabolically active membranes, the inner or cytoplasmic membrane certainly is. Yet there is only one article on the biogenesis of the cytoplasmic membranes of bacteria, and it deals largely with glycerol auxotrophs. The lack of further articles dealing in depth with the use of unsaturated fatty acid auxotrophs of *E. coli* is a notable omission. Also lacking is an account of membrane reconstitution, because a full description of membrane biogenesis will not be claimed until we have a cell-free reconstituted system that makes (or more likely, extends) membranes.

This book should recommend itself mainly to those who research on mitochondria and chloroplasts, but less so in view of its overall emphasis to those who work on bacteria. Teachers of advanced undergraduate courses should also find the book useful for reading selected topics by themselves and their students. As collections of specialist articles go, this book is a good one. Its faults are the usual ones—early obsolescence, and lack of overall balance. Its merits are the excellence and timeliness of the individual articles.

P. B. Garland

## Fossil plants in palaeoecology

*Paleoecology of Terrestrial Plants: Basic Principles and Techniques.* By V. A. Krasilov. Pp. viii+283 (Wiley: New York and London; Israel Program for Scientific Translations: Jerusalem, October 1975.) £11.00.

PALAEOECOLOGY is primarily concerned with the reconstruction of past ecosystems from palaeontological and lithological evidence. It is a vast subject that is practiced by scientists of varying disciplines, including geology, botany, zoology, and archaeology. This book fills an important gap in the palaeoecological literature, as it is the first text devoted to the use of fossil plants in palaeoecology.

After a brief introduction in which basic concepts of ecology and palaeoecology are considered, the book is divided into three major parts. The first deals with the burial, transport, and fossilisation of plant material in a variety of sedimentary environments. The second part considers the reconstruction of plant morphology from fossil remains, the environmental deductions that can be made from morphology, and the evolution of plant form. The third part discusses the reconstruction of past vegetation and environment from both fossil pollen and macrofossil remains using a variety of qualitative and quantitative approaches.

These three parts are comprehensive and thorough, with numerous well-chosen examples drawn from a very broad spectrum of both the pre-Quaternary and Quaternary literature. Much of the information is critically assembled and evaluated, although some of the views are unsubstantiated and, in places, are erroneous. For example, the widely held belief (page 144) that the *Taxodium-Nyssa* communities of the south-east US are Tertiary relics has been shown by pollen analytical work in Florida by W. A. Watts to be wrong.

My major criticism of the book is the excessive and largely unjustified use of unusual and often bizarre technical jargon. Terms such as polymeric ecological hyperspace, anthracophobic communities, palynocoenum, xylocénosis, rhizocenosis, selectogenesis, geitogenesis, endoecogenesis, and cenophyllum make reading both difficult and tiring.

In spite of this criticism, the book can be strongly recommended to all botanically-orientated palaeoecologists whether of Quaternary or pre-Quaternary interests who wish to learn more about the basic principles of their subject.

H. J. B. Birks

Newcomers to small mammalogy will find this book invaluable for it will provide them with a backcloth against which they can develop and pursue new projects. Old hands will undoubtedly be grateful to the editors and contributors for the synthesis of data and ideas that have been developed over the last decade. A book well worth reading in spite of its somewhat high price.

John Phillipson

nature

February 5, 1976

## Select Committee needs a better analysis than this

It is depressing that the House of Commons Select Committee on Science and Technology, in its latest report on research in universities (HC87; HMSO, 75p), should have marred an on-the-whole sensible and careful document by some ill-considered remarks about "a handful of highly favoured scientists" who "may influence the formulation of Science Research Council (SRC) policy". Even though the committee protests that the paper is an interim one, and that it is only asking questions and seeking out topics for further investigation, there is cause for concern that it should have gone into print with an appendix in support of this musing which can by no stretch of the imagination be called well researched.

This appendix argues that a few scientists get a lot of money. The list of 3,000 SRC grants in operation in 1973-74, totalling £53 million, was analysed, and it turns out that the top 2% of recipients get 20% of the funds (in grants of £100,000 or greater), and 10% get 45% of the funds. The mean value of grants issued by the Nuclear Physics Board (which also comprises high energy physics) is ten times that of the Mathematics Board. And people who accumulate more than one grant are more likely to be those who are already in receipt of large grants. (This rather depends on whether you believe that the 13 out of 31 recipients of big grants who receive further grants is a significantly higher fraction than the 307 out of 919 recipients of relatively small grants who land a second grant.)

The report's analysis of the trend with time shows that in 1974 almost no grants were awarded for a period of longer than three years; in earlier years 20% fell into that category. Finally, the mean grant size in nuclear and space science seems to have leapt up very significantly in 1974.

The conclusion is that there is a "concentration of large resources in a handful of outstanding individuals" which "seems to indicate at the very least that SRC policy is as much influenced by the demands of a small core of scientists as from any centralised decisions about what lines of research ought to be pursued". Nuclear physics takes a "disproportionately high percentage" of the budget; the SRC does not seem to be making any long term investments, and the increase in nuclear and space grants "would seem to indicate an increased commitment to expensive projects".

The evidence on which these conclusions or indications are based is so incomplete and selected with such ignorance of how science funding operates that the select committee should disown the report. It represents the worst sort of sociology of science in which actually talking to people is regarded as a poor substitute for computer analysis of a mass of data. Had the compiler of the report spoken to the SRC (which he or she palpably did not) before attempting to guess motives and policies, a much more profound analysis could have been made. As it is, the most glaring nonsense is talked of highly favoured individuals, "36

researchers control 23% of the budget" and so on.

Two things need to be said about this apparently charmed circle of researchers. First, some science is necessarily very expensive and requires major capital investment. To deny such expenditure simply because it is widely different in scale from that for most scientific endeavour is to condemn British science to small-minded egalitarianism. Second, the circle is not small at all. Had the committee investigated who gets the big money, they would have found that there is no room on the SRC grant application form to list co-investigators. In some cases the head of a department or a large facility (such as a radiotelescope) secures all the money under one heading, for administrative convenience, and may have 30 or more staff on the grant, pursuing independent work. In other departments, individuals go for their own grants. Thus talk of funds being concentrated among relatively few researchers is meaningless if the committee's investigations are carried out so superficially as to consign this central issue to an observation in a footnote that "research funds probably reach a wider population".

The analysis of temporal trends is equally badly under-researched. Remarks are made about the SRC's "increased commitment" to expensive projects, on the basis of a growth in the grants to space and nuclear science. This "growth" is based on a comparison between grants awarded before 1974 and still current in that year, and those awarded in 1974. But this is no comparison at all without knowledge of funding policy. If the grant committee awards money for relatively cheap research posts (assistantships and fellowships) on a three-year basis, and large capital expenditure on a one-year basis, every new year will see a carry-over from past years of relatively small sums in continuing grants, but the award of large sums for the one year. The average grant continuing into 1974 in nuclear physics was £428 a month. The average new grant was £4,359 a month. Nine hundred per cent growth or an unimportant indicator of detailed grant-giving policy? The report gives no indication that anyone tried to find out. Clearly no-one read the SRC's last annual report on the question of financial strategy. It is deplorable that such a slipshod job coupled with pure guesswork should pass for an analysis of the SRC's policy.

Science is increasingly suffering from divisiveness these days, as different groups are set against each other. If in the name of egalitarianism this fatally flawed report is accepted and used as the basis for recommendations, the committee will have to bear the responsibility for the further decline in morale among those in big science. And the clear bias against nuclear and space science pervading the report looks more than a little ridiculous when it turns out that 60% of those big grants actually go to fund engineering projects. □



Before Italy's latest government crisis, the then Minister for Research, Sr Mario Pedini, forecast that 1976 would be the turning point for Italian science. **Gillian Boucher** reports.

## Trying to make Italian scientific research useful

TO ACHIEVE one's own ministry must be a cherished hope of many a minister without portfolio. Italy's Minister for the Coordination of Scientific and Technological Research has little real power—he does not, for example, appoint the President of the CNR (National Research Council). But Sr Mario Pedini's prophecy rested on the progress of a bill being examined in Parliament for the creation of a Ministry for Scientific Research. And he spoke for many Italians when he argued for more surveillance over research. Along with the general despair over the way that both education and industry are run there has recently arisen the strong feeling that the solution lies in science—that if only academic pursuits could be programmed to the needs of the country all would be well.

In a brief history of Italian research written for a permanent commission of the Senate, A. Alberigi Quaranta summarises the attitudes which led to the current state of affairs. Italian science, stifled by the Fascists, got off to a bad start after the war. American generosity was largely to blame for this: it disguised the need for industrial innovation and provided the means for many of the brightest young researchers to emigrate. In the 1960s, while pure research was flourishing, at least in patches, the state of applied research remained pitiful. This, the argument goes, stemmed partly from the strong inclination of Italian education and culture towards theory as opposed to application, partly from the attitudes of industry, in particular of the many small firms which had not the resources for research or development, and partly from the lack of guidance on the part of the government.

Against all this there is now a violent reaction. Most of the Ministers of Research of the last ten years have in fact supported some project or other for a ministry. But the issue is now a much hotter one. Parliament has been examining two proposals for a ministry: Sr Pedini's bill, recently approved by the late government, and an extra-governmental one supported by parliamentary Christian Democrats, Communists, Socialists and Liberals.

The latter parliamentary text sees the function of the ministry as co-ordination and political direction of research: these powers, it says, would be withdrawn from the CNR. It pro-



Mario Pedini: prophecy

poses that a National Science Council should be created within the compass of the ministry, and that there should be both a national plan for research and a national fund for implementing it.

The chief difference between this proposal and the government bill lies in the government bill's proposal that the ministry, in addition, should administer research. Public bodies, and any private firm which wants public money for research, would have to present their research programmes to the ministry. The ministry would promote certain research by creating some ill-defined 'research centres'. University research would be coordinated and directed by means of a yearly conference, the composition of which may be decided by the Prime Minister. The CNR would give technical advice to the ministry and would execute its plans. Its president would be appointed by the Prime Minister, on the advice of the Minister for Research; at present the President of the CNR is appointed by the President of the Republic.

An exceedingly optimistic timetable is laid down for the yearly funding of science. Publication of the report of the conference on university research is set for this month. All research proposals will be submitted by March 1 to the ministry, which will judge them according to whether they fulfil a need recognised by the government, and whether they will duplicate

other research. The Minister will report at the end of April on the state of research and the value of the submitted projects, and the Interministerial Committee for Economic Planning (CIPE) will make its final report to the Treasury by May 31.

Reactions to the proposals in academic circles have been mixed. On the one hand many academics want to do something useful for their country, and they welcome the possibility of an end to their frustration at the lack of contact between universities and the outside world. On the other hand there is the fear, far from unjustified in Italy, that anything the government touches will grind to a halt, stalled by bureaucracy. The new enthusiasm for coordinating everything is also worrying. The universities are hardly mentioned in Pedini's bill. The sums for research they receive at present from the Ministry of Education are negligible. Pure research will clearly be in a very unhappy position if its only source of funds is through a ministry whose purpose is to administer science "in relation to the technical, social and economic progress of the country". Answering a query about his intentions for universities, Pedini has said, "Let's start with the ministry, and when it has reached puberty we'll mate them"—which is hardly reassuring.

Certain obvious questions beg themselves. Why a ministry, when a body already exists which funds and co-ordinates science, namely the CNR? Why could the CNR not forge the links between research and development, between university and industry? Part of the answer is that the CNR has tried, and failed. A total of 28,000 million lire were spent between 1964 and 1972 on twenty applied research projects; the CNR's own judgment was that most of these were unsatisfactory. Though the scientific merit of the projects was considered carefully when they were planned, there was no proper investigation of whether development would be economically feasible, or even whether there was any Italian industry in a position to use the results. The CNR, in fact, is not equipped to make decisions about economics and industry: it represents the interests of academics, with 128 of the 140 members of its Scientific Committees academics, and members of these committees also on its Administrative Board.



But the CNR, undeterred by these drawbacks and alarmed by the prospect of a ministry, is having another go at applied research. In 1972, after consulting researchers and the Office for Economic Development, it put forward 78 proposals for research projects with potential economic or social applications. By the beginning of 1975 an initial selection had reduced the number of projects to about 40, grouped into seven themes: energy (eg. solar and geothermal energy, conservation of energy); food (eg. increasing plant productivity, new sources of protein, mechanisation of agriculture, fisheries research); health (eg. preventative medicine, viruses, reproduction, biomedical technology); environment (eg. geodynamics, oceanography, soil conservation); educational theory; advanced technology (eg. telecommunications with centimetre and millimetre waves, power lasers, superconductivity, ceramics, control of air traffic); and cognitive advancement (a ragbag including criminology and astrophysics).

These projects, together with assessments of their feasibility and usefulness, were submitted to the CIPE in April 1975. The CIPE's ruling, published on October 9, was startling. The entire 'Health' programme and almost all of 'Food' were approved. 'Energy' and 'Environment' were heavily lopped; nothing remained, for example, of the projects on solar and geothermal energy. 'Cognitive Advancement' and 'Educational Theory' were rejected, at

least for the present. But most surprising, after all the trumpeting about the economic needs of the country, 'Advanced Technology' was almost completely rejected: only the project on the control of air traffic remains.

It is possible that the government has simply lost faith in the CNR's ability to know what industry needs, and prefers to wait for the Ministry for Research. This might be deduced from the extremely fuzzy explanations the CIPE report gives for its decisions. Perhaps the CIPE is concerned to prevent toes from being trodden on—the National Electricity Board (ENEL), for example, may consider research into new sources of energy its own preserve. It may also be considering the violent public dislike of multinationals, which could see the research on telecommunications, for example, as somehow pandering to the Americans.

In any case about 20,000 million lire have been set aside for the first year's research on those projects which have survived scrutiny. Owing to administrative delays it will not actually be handed out until towards the end of this year. The CNR's 1975 grant of 83,000 million lire is increased this year to 100,000 million lire, which means 3,000 million lire less for other research of the CNR—a blow at any time but especially with inflation.

Will the success of the applied projects make the loss worthwhile? Unfortunately the answer is likely to

be no. The projects suffer from the defect seen in all CNR funding, which is ultimately the fault of the way Italian universities are organised. Substantial amounts of money are divided into too many little sums, with the result that there is much duplication and nobody has the resources for a major piece of work. The practice arises because Italian universities are divided into small faculties, usually with one full professor each, which jealously maintain the barriers between themselves and other faculties. The CNR has naturally never challenged this arrangement, and nothing has changed with the new applied projects: only one quarter of the money is going to CNR institutions, some at least of which are big enough for a more flexible, interdisciplinary approach; most of the rest is destined for hundreds of university faculties.

Given this, and the fact that little thought seems to have been directed at precisely how the results achieved are to be made available to those who might use them, the projects seem likely to cause little more than a ripple in Italian economic or social life. But one thing to be thankful for is that the money is there to do something with. If a large part of it ends up financing the pure research that the academics really want to do, many would say so much the better. But when the Ministry for Research is created, money for pure research may be difficult to come by. □

## CANADA

# Getting the message across

David Spurgeon reports from Ottawa on science and the mass media in Canada

A STUDY on communication of science through the mass media confirms what Canadian scientists, science writers and ordinary readers have known for a long time: that the Canadian media do an inadequate job of making science meaningful to their public. But, surprisingly to some, it also indicates that a far greater public desire exists for science coverage than most media managers seem to realise.

The study, begun in 1973, was published recently by the Canadian

Ministry of State for Science and Technology (MOSST)\*. It indicates, for example, that science writers are willing and anxious to do a better job, but that they are hindered by the out-dated ideas and prejudices of their bosses—those who control what the media transmit to the public. At the same time, it reveals that much of the general public is just as interested in learning about science as about those other human activities that receive preferential treatment by the media; yet because of the attitudes of the media managers, this desire goes unsatisfied. The study shows how great the need is for better science coverage, for it reveals the abysmal ignorance about Canadian science on the part of those



Photo: TTC

Toronto Transit commuters: does the Canadian public at large get the science coverage it wants?

same members of the public who want to know more.

The results contained in the second volume, released to the public last month, are the outcome of three separate surveys made of managing

\*Media Impact, Volume 2, Science, Mass Media and the Public, A Research Study on Science Communication. By Orest Dubas and Lisa Martel (Information Canada; June 1975).



editors of daily newspapers with a circulation of more than 5,000, science writers and the public at large. Volume 1 contained an outline of objectives and methodology and gave an account of past research on science communication in other countries.

The public survey was conducted between March and April 1974 by the private firm, Canadian Facts: 2,000 Canadians, aged 15 and over, were randomly selected for interview. The surveys of managing editors and science writers were conducted by the authors through questionnaires. Editors of 52 of the 81 newspapers polled responded. From 176 writers (only 24 of them employed to write science or medicine full-time for daily newspapers) 113 replies were received.

Some indication of the general level of awareness of science affairs in Canada can be gained from the finding that two out of three people interviewed could not even name a single scientist. Of those who did recall one or more names, less than one in five could name more than two. And when names were mentioned, they were mostly the expected ones: Frederick Banting and Charles Best, co-discoverers of insulin, were mentioned about half the time, and—American claims for the invention of the telephone to the contrary—Alexander Graham Bell was mentioned by another third. Only seven persons among the 2,000 polled could recall the name of Dr Gerhard Herzberg, the physicist who won Canada's only Nobel Prize in the natural sciences almost half a century after Banting. Best and MacLeod were awarded the country's only Nobel Prize in medicine in 1923.

To make things worse, some of those interviewed tended to confuse Canadian scientists with those of other countries, or even with public figures who were not scientists at all. For example, 7% cited names like Albert Einstein, Louis Pasteur, Eve Curie, Charles Darwin, Jonas Salk and Alexander Fleming when asked for names of Canadian scientists. And occasionally they brought up names like Lester Pearson (former prime minister), Mme Jeanne Sauvé (former science minister), Farley Mowat (a writer on nature themes), and Fernand Seguin (a French Canadian science communicator).

When it came to Canadian scientific achievements, 61% failed to name one, 22.8% listed a single achievement, 9.7% mentioned two and 8.1% three or more. The discovery of insulin and the invention of the telephone were remembered as Canada's greatest scientific achievements, and most of the optional "projects" mentioned were really technological or engineering feats, such as the St. Lawrence Seaway or the Mirabel airport. Fewer than a

dozen mentioned the CANDU nuclear power reactor, one of the most successful designs in the world. Many of those who recalled a Canadian scientific achievement did so because they were personally involved in it, or knew somebody who was. Thus insulin, for example, was remembered because a respondent "knew the first people treated", and a heart machine because they "used it on me at the hospital."

Significantly for the purposes of the study, the mass media appear to be the major source of what little public awareness there is of Canadian science. Nearly half the respondents cited the media as their major source of information. Magazines were the main source for science news, and were preferred over newspapers and television. Radio ran a poor third, which was surprising because the State-subsidised Canadian Broadcasting Corporation provides many good science programmes; more than half the respondents, however, said they were not aware of any of the science or public affairs programmes mentioned to them. Despite their evident ignorance of the subject, 82% felt it was important to be kept informed about science, 63% were interested in finding out more about Canadian achievements, and more respondents than not (42.8 to 39.6%) thought the media were providing an adequate coverage of science.

Furthermore, without being told that the interview focused on science, the public sample rated news of science and medicine high compared with news about such other things as sport, entertainment, society, politics and foreign affairs. Medicine and health ranked third in interest after news and education, and pollution and biology rated higher than entertainment. More indicated an interest in research in the physical sciences than in sports. Crime was third last on the list of 23 items of interest, following only after a number of scientific categories. And the public even declared itself willing to give up some coverage of some of the non-scientific categories in order to learn more about science.

To anyone who has written for the public about science for years, in fact, many of the public's responses as indicated by the survey were hard to believe. One obvious weakness of the survey was the breadth of its definition of science: it included not only the natural sciences, life sciences and engineering, but also the social sciences and even the humanities, under which such topics as education, business and economics were listed. And one apparent anomaly was the finding that less than half the interested readers judged science articles in newspapers to be accurately reported. In view of their

obvious ignorance of the subject, how would they know? Three out of five, on the other hand, believed magazine articles (at least those about life sciences) to be well reported, and two out of three felt television reporting was accurate—in fact, television was perceived as the most accurate of the media, despite the fact that few magazines or television stations in Canada employ science specialists.

### The Communicators

The study's survey showed that the science writers—the people initially responsible for communicating news of science—themselves believe that the media cover science inadequately, both in quantity and quality. They, too, judged the poorest medium to be radio (which, outside of the CBC, also employs few science specialists). They felt that one of the most important obstacles they faced in reporting science was the need to cover such a wide range of topics (more than one in three wrote on at least 10 fields of science a year), but they also pointed to the difficulty in keeping stories at once simple and accurate, and the lack of time they had to research their stories. The biggest external obstacle was the traditional reluctance of scientists to communicate with the public.

A profile of the science writers revealed that 69% had a university degree, but that twice as many had training in writing and the liberal arts as in the sciences. About 40% took either supplementary training or university courses in the sciences.

The managing editors survey showed less enthusiasm for science coverage and more complacency about the way it was currently being covered than either the public or the science writers. As the authors of the study summarised it: "Editors' replies (to a section asking if they anticipated changes) were indicative of management views on most papers: that all was well with science coverage." While nearly half the papers surveyed had assigned reporters to cover medicine and health, only 22% (11 papers) had assigned a staff member to cover science. Five of these science reporters were on papers with circulations of more than 75,000. A variety of reasons were offered for not having a full-time science writer. Many editors said they could not afford it or found it cheaper to get the news from the wire services. And 29 of 33 editors who replied said their papers did not have enough staff-written science news to justify a full-time science writer—a neatly circular argument, as one critic put it.

Although both the public and the science writers who were surveyed thought it was a good idea to set up science news in a regular, easily-identi-



fied way, the editors were less enthusiastic. Between 40 and 49% of the public agreed that, when they are specifically looking for science articles in the press, they have difficulty finding them. About 68% of the science writers would like to see a weekly column on science in the press, and 44% preferred a full page weekly devoted to science. Yet among the editors, only 31% believed readers would like to see some sort of regular science feature; 70% felt that science should be presented only as items became available, and only five of 52 editors thought readers would want a full page weekly.

Perhaps some clue can be found in the educational backgrounds of the editors: less than one in three had any science courses at the post-secondary school level, and only 31% had a university degree (compared with 69% of the science writers). Although 20% of the editors reported they had had science or technical writing experience themselves, it ranged from one whose experience was "sporadic" to three with more than 20 years' experience.

The authors of the study draw the following conclusions:

"There is as yet a dilettante, hit-and-miss approach to science coverage in general, and Canadian science in particular. Even the barest informative functions of the press are not met in this area, let alone the interpretive or educative role (which is becoming more important as science affects the readers more and more).

"Often only the highlights of a scientific meeting or the outline of a project—with little relevance to impact on society are presented; in many cases by news services reporters and not by staff writers of the paper... Finally, even if good stories are written, they can still get postponed or not even used—frequently superseded by the most trivial, sensationalized items; or else buried in the paper where only the hardy will ever find them."

However, like good journalists, the authors did at least give something of the other side of the argument. One of the editors quoted, from a paper of approximately 50,000 circulation in the prairie provinces, commented: "The whole tenor of the questionnaire is so unrelated to newsroom practice that answers cannot have any validity. For example, anyone knows that any particular story has 100 facets, and to attempt to departmentalize 'science' in the modern world is meaningless. And while on any one day, no one may edit what you call a 'science' story, the next day 10 people may deal with 15 so-called 'science' stories. Go into the newsrooms and you'll find your answers. First of all, define 'science' news."

## BRITISH STEEL

# Quiet change at BSC

ALTHOUGH the spearhead of the British Steel Corporation's attempts to stem its losses and revitalise itself depend to a great extent on agreement with the trade unions to a reduction in the total labour force (some 220,000 people at present), a step forward in rationalising its research and development was taken recently with the minimum of fanfare and publicity. Quite simply, the BSC now has a Chief Scientist and a Controller of R and D, in the true Rothschild tradition of the "customer-contractor principle".

The annual research budget of some £13 million may not seem much by comparison with the corporation's losses, which are now running at about £8 million a week, but when the BSC is properly on its feet again, it will undoubtedly profit from the more razor-edged research arrangements that now seem in prospect.

As from the beginning of last December, Dr Robert S. Barnes, until then Director, Research and Development, became Chief Scientist, and Mr James Mackenzie, who had been Technical Director of the General Steels Division, became Director, Research Laboratories. This means, essentially, that Dr Barnes says what he wants done and Mr Mackenzie establishes that it can be done and sees that the work is carried out.

At present the two men are sizing their new jobs up and it will be a few months before they finally sort out precisely how their responsibilities should be divided. It is fairly certain, for example, that Dr Barnes will have under him a team which will be responsible for investigations into the cost-benefit of research done by the BSC and for techno-economic analysis. Until December, research was carried on within the corporation's four divisions and at three "corporate laboratories" which used to make up the old British Iron and Steel Research Association, BISRA. This somewhat decentralised system has its roots back in the days before British Steel was nationalised, and it is no secret that there has been some overlap and duplication of work, which admittedly has been decreasing over the years.

The corporation has no plans to close any of the laboratories or to cut its spending on research, and any redundancies that may occur will be part and parcel of the BSC's overall labour strategy. The changes do mean, however, that laboratories will lose some of their autonomy under Mr Mackenzie, who will be based in Teesside in North-east England. And there

seems little chance of major new research avenues being funded with new money in view of the corporation's perilous financial situation.

These changes in research and development do little, of course, to help the corporation with its immediate problem of turning itself into a viable entity. Since nationalisation in 1967, BSC's capacity of some 27 million tonnes a year has been maintained while the number of individual steel plants has been reduced from 40 to 30, and the corporation's basic aim is to produce some 80% of a projected output of 35–37 million tonnes (in the mid-1980s) in just five major integrated steel complexes—two in Wales producing 9.5 million tonnes a year between them, two in North-east England with a combined output of 16 million tonnes and a Scottish complex (4 million tonnes). In these giant plants, ore will be turned into a finished product on a single site. The remaining 20% of the output will be mainly of special steels, and this activity will, as at present, be centred on Sheffield.

Up-to-the-minute though this may sound, BSC will be doing no more than the Japanese and others have already achieved: the Nippon Steel plant at Fukayama, for example, has an annual output of some 16 million tonnes, and that at Dunkirk in France produces about 8 million tonnes a year. It is widely agreed that a really viable steel plant must be sited near a deep-water terminal (thus eliminating the relatively very high cost of transporting ore by rail) and should have an output of several million tonnes a year (thus opening up the advantages of ore transportation in 250,000-ton bulk carriers).

**Roger Woodham**



Photo: Harrison & Laking

Robert Barnes, Chief Scientist at BSC.



## US ENERGY

# Saving without changing

*An aggressive energy conservation programme would enable the United States to meet all its energy requirements for the next 25 years without recourse to environmentally destructive new sources of supply, according to a provocative study published this week. Colin Norman reports from Washington.*

FULLY half of the energy now used in the United States is wasted, and massive savings are possible without major changes in the American way of life, according to a study written by Denis Hayes, a former energy adviser to the Governor of Illinois. The study was published by the Worldwatch Institute, a relatively new Washington-based research organisation whose previous analyses of global problems, such as food supply, have attracted considerable public attention.

The conclusions reached by Hayes run counter to the conventional wisdom that energy consumption is so closely correlated with standard of living that major reductions in energy use would precipitate severe economic dislocations and cause living standards to drop drastically. Hayes says, however, that his conclusions rest on the assumption "that lifestyles will change only cosmetically—that Americans will continue to travel as many miles, keep their homes just as warm, operate as many appliances, and eat what they now eat". Moreover, he argues that a strong energy conservation program would actually increase employment and "save consumers billions of dollars a year".

Hayes told a group of reporters last week that he reached his conclusions about energy conservation after becoming convinced of the potential difficulties in opening up new sources of supply. Noting that economic and technical problems have caused some of the leading industrial contenders to drop out of the effort to develop oil shale, that increased use of coal would create unacceptable environmental pollution, and that nuclear energy is beset with troubles, he said that "source after source is becoming increasingly less optimistic". Moreover, since oil and gas reserves in the United States are dwindling fast, Hayes suggested that "we are consuming energy like a childless society", rapidly depleting energy reserves "with little regard for the future energy needs of our children".

The American way of life is "rife with opportunities to conserve energy", Hayes says in his report.

- The major opportunities lie in transportation. Hayes estimates that some 42% of total energy consumption in the United States is accounted for, directly and indirectly, by transportation; he reckons that half of that amount could ultimately be saved. His suggestions include gradually tripling the mileage performance of vehicles, mostly by reducing the weight of automobiles; greater use of public transportation and car pooling; and shifting freight from trucks to more efficient means of transport such as railroads and waterways.

- Stricter insulation standards for new buildings and increased insulation of existing structures, coupled with greater use of solar energy for heating and air conditioning, could save as much as 16% of present consumption, he calculates.

- Technical improvements in agricultural production and changes in the way food is packaged and sold could save another 5%.

- Improvements in the efficiency of electricity generation, through more use of waste heat and better generating technology, could save 3% of present energy consumption.

- Finally, greater use of recycling and elimination of unnecessary packaging could save 4% of current energy needs.

Few of those suggestions are new. In fact, most of them were aired in a massive study of energy policy options for the United States, published in 1974 by the Ford Foundation's Energy

Policy Project. The catch, of course, is that the road between devising an energy conservation programme and implementing it is paved with political problems. Though Hayes notes in his study that such problems exist, he offers few suggestions for overcoming them.

To achieve the level of energy saving which his study suggests would require considerably increased government regulation of various industries, use of tax incentives for increasing efficiency and other similar devices. Most of those measures would be opposed by a powerful coalition of industrialists, energy interests and financiers.

But perhaps the chief political problem is that the greatest single incentive for energy conservation would come from greatly increased energy prices, which are still comparatively low in the United States. Though he does not discuss prices at length in his study, Hayes said last week that he would personally favour much higher prices than even a free market would produce (natural gas and oil prices are now controlled to some extent by the federal government). But he added that if prices are allowed to rise, an income distribution scheme would be required to prevent the burden falling on the poor. That, of course is a politically explosive issue. Last year, Congress balked at decontrolling oil and gas prices, partly because of the suspicion that oil companies would be able to reap greater profits if controls were removed, but mostly because of the fears of a voter backlash provoked by rising energy costs.

## Boost for biomedical research

CONGRESS last week handed President Ford a stunning political defeat when it voted, by a surprisingly large margin, to ram a budget bill for the Department of Health, Education and Welfare (HEW) into law over his objections. By its action, Congress has lifted the sagging budgetary fortunes of the National Institutes of Health, for the bill contains a large helping of money for biomedical research—considerably more than Mr Ford wants to spend. There are still a few political battles to be fought before NIH can spend the money, however, and the exact amount and the timing of its release by the Treasury are not yet certain.

The HEW budget bill—which applies to the fiscal year which began more than 7 months ago—has been tangled in a struggle between Congress and the Ford Administration over spending priorities. The supporters of the overriding action have

argued that since Congress had already cut the defence budget, an increase in the HEW budget would not add to total government spending, but merely shift priorities from defence to domestic programmes.

As far as NIH is concerned, Mr Ford had proposed that it should receive slightly less money this year than it received in the 1975 fiscal year. The bill passed by Congress, however, will increase NIH's budget by some \$200 million.

Mr Ford might still be able to delay the expenditures and perhaps, reduce them a little. In the next few days he will formally propose to Congress that some of the money should not be spent this fiscal year; Congress will have 45 days in which to act on that proposal. That means NIH will not get its budget sorted out until three quarters of the fiscal year has gone by. Another battle will then begin over HEW'S Fiscal year 1977 budget.

## BRAZIL

Two Brazilian ecologists are making progress in their fight to save the "mico"—a small, round-faced, long-tailed monkey of the genus *Cebus*—from extinction. A generation ago, when Rio de Janeiro's famous Copacabana beachfront neighbourhood was a quiet, distant retreat for fishermen and weekend trippers, with hardly any permanent residents, countless thousands of micos played in the branches of trees that proliferated in that district. Now, with Copacabana turned into an overpopulated jungle of high-rise apartment buildings, almost no micos are left in Rio. (Fewer than 1,000 are known to exist in the whole of Brazil.)

For several years, however, ecologists Adelmar Coimbra Filho and Alceu Magnanini of the Rio State Engineering and Environment Foundation, have been battling to prevent the mico from disappearing. They started out by keeping a few micos in the safety of their back yards and encouraging them to breed. In 1972 they obtained a foreign grant and created a biological preserve in Rio's still-untouched Tijuca Forest. Today the mico population there has grown to 58.

The prospects for keeping the mico from becoming extinct are now better than ever before. Brazil's federal Land Reform and Colonisation Institute has promised Coimbra Filho and Magnanini that it will expropriate a wooded tract of land north-east of Rio—a former natural habitat of the mico—and turn it into a restricted-access biological area where the principal priority will be to preserve this species of monkey.

Magnanini, who at one point in his career was ready to pronounce the mico extinct (until his partner, Coimbra Filho, happily discovered one), says the mico had the misfortune to be best adapted to Brazil's humid Atlantic coastal region—where development of the country has been most intense.

● Brazil's Health Ministry has released partial results of its most intensive

survey to date on the incidence of Chagas Disease, and the picture is not very bright. Researchers found the ailment prevalent in 1,238 of 1,750 counties that were surveyed (Brazil has nearly 4,000 counties). These results led to estimates that 8 million people in this country may have the disease, either in its latent or acute form.

Chagas Disease is a Latin American malady for which there is no known cure. It is caused by a protozoan parasite known as *Trypanosoma cruzi* and is transmitted by an insect known colloquially as the "barbeiro" (Portuguese for "barber bug") or "chupao" ("sucker"). Chagas Disease, which attacks the heart, causes long term debility, often resulting in death. Most fatalities are among people 25-50 years old who come from rural areas.

The disease is transmitted in a bizarre way: "barber bugs" living in the thatched roofs or mud-brick walls of peasant farmers' shacks come out at night and bite people, usually near their eyes. Then the insect defecates into the bite, sending the dangerous parasite directly into the person's bloodstream. The Health Ministry survey showed that Chagas Disease is further spread through unsupervised blood transfusions—even in seemingly modern, advanced cities such as Belo Horizonte and Sao Paulo, where there are large colonies of farm migrants.

"In Brazil, most cases of Chagas Disease go unrecognised, because of a lack of good medical care in rural areas," declared Dr J. Romeu Cancado, head of the medical clinic at the Federal University of Minas Gerais Medical School. He added that the problem of Chagas Disease has been further complicated by failure to include a detailed study of the disease in leading Brazilian medical schools. Most medical researchers at present doing work on Chagas Disease are in fact non-Brazilians.

Asked about prospects for finding a cure for Chagas Disease, Dr Cancado said that several drugs had been pre-



sented recently as cures for Chagas Disease, but most had turned out to be too toxic or ineffective in humans.

● Brazil is seriously determined to cut its petroleum consumption (it had to import four-fifths of its crude oil in 1975, at a cost of around US \$3,800 million), and President Ernesto Geisel has asked petroleum researchers to develop an efficient Brazilian product that would contain 20% alcohol.

Brazil has great potential for producing alcohol. It is the world's biggest cane-sugar grower, and it has vast plantations of cassava root and sweet potatoes, which are thought to be alternative sources of extractable alcohol. The nation's present rate of alcohol production is 270 million litres a year. Based on figures for petrol consumption, Brazil would have to boost alcohol production to 3,000 million litres annually to meet the 20% goal.

Brazilian gasoline now contains about 5% alcohol. Some say that the alcohol mixture can be increased to 20% without lowering the efficiency of automobile engines, but Mario Gellini, an engineer with the Brazilian subsidiary of the Ford Motor Company, says that 15% is the maximum amount of alcohol that can be tolerated if cars are not to run badly.

**Bruce Handler**  
Rio de Janeiro



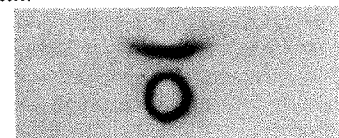
### Competition 5

As correspondents continue to point out, *Nessiteras rhombopteryx* is an anagram of "Monster hoax by Sir Peter S." or "Yes both pix are monsters, R." (for Rines). A prize of £10 is offered for the best anagram of any scientific concept. Closing date for entries is March 10.

FOR **Competition 4** readers were invited to submit about 100 words culled from the pages of *Nature* 100 years hence. Entries from F. P. Hughes and S. Gilbert deserve special mention, but the winning entry came from R. Ternbach, of Cambridge, Massachusetts, who receives £10 for this submission:

The Lunar Observatory for Orthomolecular Nebulosity has surveyed one of the twelve equally spaced peculiarities recently shown to surround the galaxy at cosmological distances. Optical plates exposed through the moonstation telescope show a ring (head) with an arc (horns) above.

Gas clouds are ejected from twin singularities (nostrils) in the head at a rate of  $10 \text{ min}^{-1}$  (breathing rate). Glitches in the ejection rate (snort bursts) have a mean inter-snort interval of 1 sec. Absorption spectra of snort gases suggest a molecular composition strikingly similar to bovine breath.



**Fig. 1** Appearance of the peculiarity on optical plate made at the Lunar Observatory for Orthomolecular Nebulosity.

## SWEDEN

# Making sense of senselessness

*As progress falters in the drawn-out second round of the Strategic Arms Limitation Talks (SALT), concern is also growing about the implications of large-scale environmental warfare. But popular attention is often diverted away from both the true state of present technological developments and the strategically valueless damage which the military use of existing techniques has already done. From Stockholm, Wendy Barnaby reports.*

WHEN two such prestigious organisations as the Royal Swedish Academy of Science and the Stockholm International Peace Research Institute (SIPRI) collaborate to produce a public statement on a topical issue, it is not surprising that it should attract a lot of publicity. But press reports of their predictions about war and the environment, which are contained in the latest edition of the Academy's journal *Ambio*, have generally been both inaccurate and misleading.

The journal describes the damage done to the biosphere by war, and the ways in which natural environment phenomena could be manipulated for belligerent purposes. It also discusses some of the international negotiations to draw up treaties prohibiting modification of ecological systems for hostile ends. Most of the publicity surrounding the issue has concentrated on articles which document the worldwide spread of all types of sophisticated weapons, describe the likely results of a nuclear war in Europe, and catalogue the possibilities of weather manipulation.

Press accounts of the prospects for environmental warfare have dwelt on seeding clouds, modifying hurricanes, oceans, earthquakes and climates, and tampering with the ozone layer, without mentioning what *Ambio* stresses: that the techniques for doing these things involve many practical difficulties and are at the moment "theoretical and speculative" only. Geophysical modification as a weapon system is certainly not just around the corner, but the presumption is that the public has the right to know which stage in the sequence has been reached: a *fait accompli* is harder to oppose than something not yet completed.

The global proliferation of modern conventional weapons suggests that more attention might be paid to the damage they can cause. Dr Malvern Lumsden, a Research Fellow at SIPRI, describes the impact of strategic bombing in the Second World War, for example of Berlin, and of the bombing

of South Vietnam by the United States. He concludes that the level of social organisation (which is different from the level of "development") of the bombed population is the prime determinant of its ability to withstand environmental stress. This conclusion is interesting in the context of the environmental stress that urban guerillas seek to inflict through the use of terror—a topic on which the journal might have shed some light by considering which factors most often influence the outcomes of sustained campaigns of kidnapping, hijacking and attacks against embassies.

Apart from anticipating future weapon systems, the press could also publicise the extent of the damage caused by those presently deployed. In another article, a former US marine officer, Professor Arthur Westing, describes the three prongs of American tactics in South Vietnam between 1965 and 1973. Professor Westing, presently a botanist who visited SIPRI as a researcher in 1975, details the effects on the country's ecology of high explosive munitions, herbicides and land-clearing tractors ("Rome ploughs"): the instruments used by the USA to make the land inhospitable to enemy guerillas. One quarter of the land area of the entire country was covered by B-52 crater fields alone. One tenth of the land area—about 1.7 million hectares—was sprayed once or more with herbicides. And about 2% of the land area was cleared by Rome ploughs, 33,000-kg armoured tractors whose blades can split and topple trees of almost any size. These destructive techniques proved to be of only doubtful military value, but Professor Westing forecasts that future combatants will intensify their use in order to raise their military pay-off. The result will be even more desolation than was caused in South Vietnam.

Destruction on the scale implied by present levels of armaments can, of course, only be supported by enormous resources. Another SIPRI Research Fellow, Ronald H. Huiskens, calculates that world military expenditure totalled \$210.300 million in 1974, and that the raw materials used for global military purposes are at present worth about \$250.000 million—the equivalent of the combined gross national products of the 65 countries of Latin America and Africa. That natural resources are used to desecrate the environment in which they were formed represents just one of the many ironies characterising the present ridiculous levels of militarisation. □

## CERN

# II on target

*The Geneva-based European Organisation for Nuclear Research (CERN), in which 12 European governments participate in a collaborative programme of subnuclear physics research, largely through teams of visiting scientists, has two particle accelerators that have been operational for many years. Peter Collins reports on the super proton synchrotron (SPS) now under construction*

THE meeting of the CERN Council just before Christmas marked the end of an era with the retirement of Professor W. Jentschke following his five-year term as Director General of CERN I, the original CERN laboratory. Since January 1 of this year the two CERN laboratories—CERN I and the one to house the SPS, CERN II—have been combined. Instead of two separate Directors General, the whole complex has two men working side by side: Dr J. B. Adams, as Executive Director General, is responsible for everything concerned with management, while Professor L. van Hove of Belgium fills the new appointment of Director General responsible for research.

Interest at CERN inevitably centres, however, on the 400-GeV SPS. Unlike many (and perhaps most) such building projects, its construction is moving ahead on schedule and within its estimated budget. The last of 744 bending magnets in the 6.9-km ring was installed a month or so ago to coincide with the meeting of the CERN Council. According to Dr Adams, who was the Director General of CERN II, there have been few serious problems during construction of the new machine.

Apart from the apparently inevitable delays arising from late delivery of components, only one major difficulty seems to have arisen. This was the discovery, early in 1975, of serious deterioration in the insulation of a number of the magnets that had already been installed. Urgent and intensive detective work showed that the failure was always in the same area of the coils; it was found to be caused by the use, on the ends of cables, of a cleaning fluid that contained a high proportion of phosphoric acid. Where this had not been completely cleaned off, the acid attacked the resin used for insulation, and had the effect of continuing its "curing". The result was that the resin hardened, shrank, and eventually cracked, allowing the acid to get through into the glass cloth used for the inner insulation, and thence to the

THE annual review of the Christian names of the "top children" whose birth are announced in the London *Times* appeared as usual in the New Year. For the boys, James, Thomas and Nicholas headed the list, with, for girls, Sarah, Emma and Alexandra in the corresponding positions. This year the list has stimulated an interesting correspondence on the sociological problems relating to the choice of what are perhaps more accurately described by the American term "given" names. No *Times* reader apparently calls his child Tracy or Charlene, though there is a growing tendency to saddle girls with classical names like Corinna, Flavia, Gratia and Xanthe. One writer, from the Savage Club, calling the list "astonishingly class distinctive", recommended the introduction of a Christian Name Discrimination Act, banning socially-divisive names and having, like the French, an approved list from which names must be drawn. This last course has given difficulties to Breton nationalists who wish to use names of folk heroes who were not recognised by Napoleon.

Names may be as interesting to the parasitologist as to the sociologist. In 1939 I became involved in the problem of lousiness in English children. Before the war official statistics suggested that the head louse was almost extinct, for only one or two per cent of children were detected with the parasites by the officials of the School Medical Service in their routine school inspections. However, when town children were evacuated to the country to escape the risk of bombing in September 1939, far more than had been expected were found to be verminous. The Board of Education, as it then was, defended its statistics, and suggested that the children had become infested during the long summer holiday. Political arguments arose. Socialist education committees in towns accused Tory reception areas of exaggeration. No one seemed able to produce the facts

which had led to what was rapidly becoming an ugly situation, particularly as there was as yet no enemy bombing to distract people's attention.

As no one else seemed to be trying to find out the facts, I approached

## Names to remember



KENNETH MELLANBY

the Board of Education with a plan. To the Board's credit, I was given every encouragement and £200 to investigate. I found that, in our cities, over half the girls between 5 and 12 years old were lousy, and that a third of the boys were similarly affected. There was no evidence that the rate of infestation rose during the holidays. It was clear that most infestations were not being detected at routine inspections.

These results were all published with the approval of the Board of Education and the Ministry of Health, though they were clearly critical of work of those government bodies. My paper was censored only

in very minor ways. I was asked to omit a section where I identified the incidence of infection in children who were registered as belonging to different religions and different Christian denominations. I agreed that this information might exacerbate inter-sectarian quarrels. But I was also asked to cut out a section on Christian names, which was thought to be too frivolous for war-time publication, and I think that the information may now be revealed.

In one city which had a particularly high rate of lousiness (70 per cent of all ten year old girls) I noticed that children named after film stars, children with saints' names and children with the names of the Royal Family were the most likely to be lousy. Marlene (often spelt Marleen), Shirley, Bernadette, Margaret, Rose, Marina—they always warned me in advance what to expect.

This, of course, brings us back to the sociological field. Lousiness tended to be highest in large families, where reinfestation from siblings even after successful treatment was common, and where individual attention by the mother was less effective than when she had fewer children to care for. It has been suggested that children named after film stars or royalty come from families with less of a tradition than those where the same few names are used generation after generation, and where parental care may be greater. This may be the explanation. But I suggest that anyone studying sociological problems relating to names bears these observations in mind. During the war I found them useful. Although the results had to be confirmed by individual examinations, I could make a good estimate of the lousiness of a class of school children simply by reading through the register. As the head louse is now staging a comeback in some British towns, it will be interesting to see whether it still prefers to infest the holders of any particular names.

coils themselves. The insulation system itself then became a conductor.

Every one of the 300 or so magnets already assembled was examined, and dismantled, repaired or rebuilt where necessary. As an extra precaution, further insulation in the form of two layers of kapton foil was wrapped round each coil, and it was found possible to do this with those magnets which had not deteriorated, but had already been installed. Every one of the 744 magnets in the ring has been treated in this fashion.

The fact that nearly 300 of these big

magnets were rebuilt within the SPS Assembly Halls, without delaying the very strict construction schedule and using CERN's own staff and facilities, is an indication of the efficiency and skill with which the whole project is being handled. Tested under conditions with peak fields equivalent to the 400-GeV energy level for which they are intended, the magnets have shown no further failing, nor do there seem to be any serious problems with any of the other components.

Apart from some delays in delivery of magnets for the beam transfer lines

to the experimental areas, the rest of the construction is well on target, and the accelerator is expected to be operational in the latter part of 1976. This is perhaps no more than was to be expected from an establishment that runs as sweetly as CERN. To many it is an object lesson not only in international scientific and technical cooperation but in the noticeably pleasant atmosphere and evidently high staff morale as well. It could well be studied by certain international agencies of the UN family a few miles away in Geneva itself. □



# correspondence

## Proceedings against professor

SIR,—In *Nature* recently under the heading 'Anti-nuclear critic faces dismissal' (October 23, 1975, page 636) it was maintained that Professor Jens Scheer is "head of a university department" and is threatened with dismissal apparently because he criticises the construction of nuclear power stations. Both statements are incorrect.

(1) The "head" of our department is elected democratically each year by the professors, students and technical personnel. Professor Scheer has never held this office, which is at present occupied by Horst Diehl.

(2) It is true that various disciplinary proceedings have been initiated against Professor Scheer but the reason for these measures is neither his criticism of the West German nuclear programme, which is shared by a number of his colleagues against none of whom have any such proceedings been taken, nor simply his membership of what we consider a rather insignificant Maoist group.

Your readers might infer from the article that the West German authorities disregard individual rights and use improper methods in order to silence their critics and that the true reasons for the initiation of disciplinary proceedings against Professor Scheer are not those which had been stated officially. Such an inference would be wholly unfounded; readers should be aware of the following facts.

On two separate occasions, Professor Scheer was involved, with others, in violent activities at the University of Bremen: in October 1973 a meeting of Christian Democrat students was broken up by force, and in September 1975 a press conference of the Students Committee was pelted with eggs and tomatoes. In both cases the university took out disciplinary proceedings against Professor Scheer. The earlier case, in which the Rector imposed a fine on Professor Scheer, is under appeal; the latter is still under consideration. Professor Scheer's action in 1973 also led to the District Court of Bremen sentencing him to three months imprisonment (suspended) and a fine of DM3,000. This too is under appeal.

Action has also been taken against Professor Scheer by the Senate of the Free Hanse Town of Bremen under the provisions of the Bremen Civil Servants Law. Since December 9, 1975, he has been under suspension on half

pay as a result of his alleged anti-constitutional activities.

Yours faithfully,  
STEFAN VON AUFSCHNAITER  
(Konrektor of the University)  
SIEGFRIED BOSECK  
HORST DIEHL  
WOLFGANG DREYBRODT  
DIETER VON EHRENSTEIN  
KLAUS HAEFNER  
PETER RYDER  
HELMUT SCHWEGLER  
WOLF SIEGERT  
(Professors of Physics)

University of Bremen,  
Bremen, West Germany

## Naming the blue-greens

SIR,—Recently, in several publications, organisms hitherto known as blue-green algae have been called "blue-green bacteria". It is appropriate to consider the popular name of the Cyanophyceae in a scientific journal since words, especially those used in science, should have clearly defined meanings and should not be subject to passing whims or personal preferences.

Cohn, more than a century ago, associated the apparently non-nucleate blue-green algae with the bacteria in the division Schizophyta (*Beitr. Biol. Pflanz.*, 1, 141–207; 1875). Chatton subsequently distinguished the characteristic cellular organisation of these types of organisms as "procaryotic", (*Titres et travaux Scientifiques*, Sète, Sottano, 1937), and on this basis Stanier and van Niel proposed a major taxonomic dichotomy among living organisms, establishing the Prokaryota and Eukaryota (*J. Bact.*, 42, 437–466; 1941). Although the Prokaryota are essentially the same as the Schizophyta, rules of priority have evidently been waived and this distinction has become generally accepted. The eighth edition of *Bergey's Manual of Determinative Bacteriology* (Williams and Wilkins, 1974) refers to "the blue-green algae" as "allied to bacteria and at a level of equal importance . . . to the remainder of the Kingdom" (that is the Prokaryotae). But this is not to say that they are to be considered as bacteria. If we were now to start referring to cyanophytes as 'blue-green bacteria', we would implicitly synonymise the words 'prokaryota' and 'bacteria'. To what useful end?

It is generally accepted that living organisms should be named according

to one of three texts: the International Rules of Botanical, Zoological and Microbial Nomenclature. If a creature is to be 'moved' from one kingdom to another, its whole taxonomic status has to be reviewed and may have to be revised. If those of a whole class were to be involved, this would entail a lot of extra, and in my opinion, unnecessary work and headaches.

The word 'algae' has no clearly defined taxonomic meaning. It is a useful and convenient term for most non-vascular aquatic plants capable of photosynthesis with  $O_2$  evolution. If the Cyanophyceae were to be shifted, lexicographically, from the algae to the 'bacteria', it would be necessary to change many other generalisations to conform with this transfer. For example, the primary producers which evolve  $O_2$  would include 'bacteria' as well as green plants. The photosynthetic 'bacteria' would include aerobes as well as anaerobes, would contain either chlorophyll *a* or bacteriochlorophylls in thylakoids or associated with other subcellular elements, and would fix  $CO_2$  either with or without  $O_2$  evolution. Many of the seaweeds which often dominate tropical shores would have to be regarded as 'bacterial' growths. What would we gain by such verbal changes?

Unlike non-photosynthetic prokaryotes, blue-green algae rarely cause disease or decay, and thus relatively little money is spent on them. (Anyone who questions this should compare the budget of a bacteriology department with that devoted to the study of blue-green algae.) But although cyanophycologists are in a less fortunate position than bacteriologists when it comes to funding for research, personal remuneration, or public support, they should not seek to better their lot by calling themselves blue-green bacteriologists. Scientists should not use words misleadingly for base ends. In spite of the fairly evident and generally accepted phylogenetic homogeneity of the Prokaryota, the distinction between the classes are worth retaining. Although we may now handle them by similar technologies, yeasts are fundamentally fungi, cells from tissue cultures remain basically animal or plant cells, and blue-green prokaryotes are, by accepted standards, algae.

Yours faithfully,

RALPH A. LEWIN  
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# news and views

## Spacing out the histone genes

from Benjamin Lewin

THE organisation of clusters containing many identical copies of a gene(s) is among the more intriguing features of eukaryotic DNA. The first such cluster to be identified was that of the genes coding for ribosomal RNAs. Assisted by the ready isolation of ribosomal genes in the form of the amplified nucleolar DNA of *Xenopus laevis*, analysis of the rDNA has shown that the pattern of organisation takes the form of a repeating series of transcription units, each representing a precursor molecule containing the sequences of both major rRNAs, adjacent units being separated by a region of DNA that is not transcribed. The transcriptionally inactive DNA separating the sequences coding for the precursor RNA is often termed the non-transcribed spacer. Because the precursor is itself somewhat larger than the total length of both mature RNAs (the length of the excess varies with the organism), the part of the precursor that is discarded is sometimes known as the transcribed spacer; its sequence is invariant and it most probably functions at some step after transcription. One of the most striking features of the rRNA gene cluster is that in spite of mutational pressure the copies of each gene seem to be identical. This implies that some mechanism must exist for suppressing variation among the gene copies and this may be the role of the non-transcribed spacer.

The histone genes comprise another system of clustered repetitive genes. The histone genes of the sea urchin are all repeated several hundred-fold in the genome, and similar repetition is also found in other organisms to a varying degree. Because their base composition differs from that of the bulk of the genome, the histone genes can be isolated as a satellite fraction of distinct buoyant density. This in itself suggests that they are clustered into one or a small number of groups. Two types of arrangement might be possible—separate clusters each representing many copies of one of the five histone genes or a single type of cluster containing all five genes. Two recent series of experiments have demon-

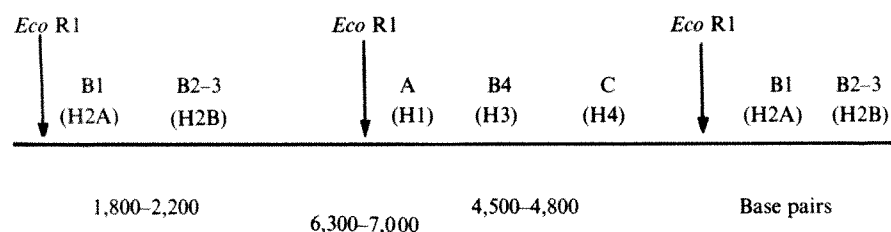
strated the existence of a single type of repeating unit and also have implied that it may possess a spacer region(s) longer than the total coding length of the five genes.

These experiments were made possible by the fact that the restriction nucleases *Hin* III and *Eco* R1 make a limited number of cuts in what can be inferred to be a simple repeating unit. Because such a large amount of histone DNA is present in the sea urchin genome, its cleavage products can be identified directly without the need for extensive purification of the starting material. Kedes *et al.* (*Cell*, **6**, 359–369; 1975) showed that when the DNA of *Strongylocentrotus purpuratus* is treated with the enzyme *Hin* III, a single major fraction is present upon centrifugation through a sucrose gradient; this represents histone DNA and sediments at 17S. Treatment with *Eco* R1 nuclease generates two major fractions, sedimenting at 14S and 11S. Starting with partially purified histone DNA (5–30 fold enriched), Weinberg *et al.* (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 4815–4819; 1975) characterised the restriction products upon acrylamide gels. *Hin* III generates a single fragment with a mobility suggesting a length of about 6,300 base pairs; *Eco* R1 produces two fragments, with mobilities corresponding to 1,800 and 4,500 base pairs. Only these fragments hybridise with histone mRNA; their gel mobilities are consistent with the sucrose sedimentation rates. The model immediately suggested by these results is that histone DNA consists of a single type of repeating unit, which happens to have a single site recognised and cleaved by *Hin* III and to have two sites that are subject to attack by *Eco* R1 nuclease.

By mixing *S. purpuratus* DNA cleaved by *Eco* R1 with the *Eco* R1-treated DNA of plasmid pSC101, Kedes *et al.* (*Nature*, **255**, 533–537; 1975) isolated hybrid plasmids carrying random parts of the sea urchin genome. Hybrid plasmids carrying histone genes were identified (after cloning in *E. coli*) by hybridisation with labelled histone mRNA. Two plasmids carrying histone

genes were characterised: pSp2 includes a DNA fragment estimated at 4,400 base pairs by gel electrophoresis and pSp17 carried a fragment of 1,700 base pairs. When Kedes *et al.* (*Cell*, *op. cit.*; 1975) investigated the nature of these histone gene sequences, they found by electron microscopy of heteroduplexes that pSp2 and pSp17 are homologous only along the 8,700 base pair length of the pSC101 sequence itself. The contours of the single strand loops give more accurate assessments of the lengths of the eukaryotic fragments, 4,800 base pairs in pSp2 and 2,200 base pairs in pSp17. Hybridisation experiments confirmed that the pSp2 plasmid carries the sequence of the 14S fragment cut from histone DNA by *Eco* R1 whereas the pSp17 plasmid represents the 11S sequence. The lack of homology between the 4,800 and 2,200 base pair sequences supports the idea that the repeating unit of histone DNA has been cut into two parts.

Which histone genes are represented on these two parts of the repeating unit? Histone mRNA can be isolated from the polysomes of developing sea urchin embryos as a 9S fraction and the mRNA of *S. purpuratus* has been separated into several classes on acrylamide gels. Two of these classes, A and C, have been shown to code for histones H1 and H4 respectively (Levy *et al.*, *Cell*, **4**, 239–248; 1975). The classes B1, B2, B3 and B4 together code for H2A, H2B and H3; the individual classes have not been equated with particular histones, but comparison with the template activities of the corresponding classes in another sea urchin, *Lytechinus pictus*, suggests tentatively that B1 may code for H2A, B2–3 for H2B, and B4 for H3. When these messenger fractions were hybridised to the hybrid plasmids Kedes *et al.* found that A, C and B4 annealed exclusively with pSp2 whereas B1 and B2–3 annealed only with pSp17. Using histone mRNA fractions separated in the same way, Weinberg *et al.* obtained precisely the same results with the *Eco* R1 histone DNA fragments separated on acrylamide gels. This suggests a map for the histone genes:



Two features are implicit in this map; it represents a regular repeating structure containing all five histone genes; and it must have appreciable spacer regions since the total length needed to code for all five histones is only 2,100 base pairs. This organisation obviously raises the question of whether the histone genes are transcribed as a single unit, presumably cleaved later into the individual messengers that represent the only form in which template activity for histones has been identified. Histones H2A, H2B, H3, H4 are found in equimolar amounts in chromatin, which makes their coordinate gene expression an attractive model, but H1 is found in smaller amounts. No precursor to histone mRNA has been identified, but it is interesting that such a model would imply that at least some of the spacer is transcribed. What is known about the sequences of the histone messengers and genes suggests that there cannot be much variation in the copies of each gene; probably they are all identical. This is subject to the caveat that although H2A, H2B, H3 and H4 all display unique amino acid sequences, some variation is found in H1, which may therefore be represented by more than one type of gene; how the H1 genes might be arranged and controlled within the repeating cluster is a matter

of some interest. In contrast to the apparent homogeneity in the copies of each histone gene in a given sea urchin species, Grunstein *et al.* (*Cold Spring Harbor Symp. quant. Biol.*, **38**, 717-724; 1973) noted that there is only limited homogeneity between the corresponding histone genes in different sea urchin species (in spite of the common amino acid sequences of the corresponding proteins); this emphasises the evolutionary stress that must be placed on conservation of sequence within a species. What is responsible for the maintenance of identity among the gene copies? It is tempting to speculate that some of the regions which do not code for histones might represent non-transcribed spacer, in which case a common type of structure might be proposed for both the rRNA and histone gene clusters, with a non-transcribed spacer perhaps implicated in helping to suppress variation. Of course, it is also possible that the unidentified regions of the histone gene cluster may code for other proteins or represent transcribed spacer. Important future experiments therefore will be to define completely the location of each histone gene in the repeating unit and to investigate the sequence organisation of the remaining sequences, and to determine whether they represent transcribed or non-transcribed regions.

The scene for the recent reports was set a few years ago by De Maeyer and his colleagues who boldly applied RNA, extracted from either mouse or monkey cells which had been induced to synthesise interferon, directly to heterologous cells and showed that the recipient cells were able to make murine or simian interferons, respectively (De Maeyer-Guignard *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1203-1207; 1972). The amount of interferon made was quite small (not more than 30 units) perhaps due to the probable inefficiency of exogenous mRNA when applied directly to cells.

The obvious extension of this work has now been reported by De Maeyer and his coworkers (Thang *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3975-3977; 1975) who have translated RNA extracted from induced mouse cells in a wheat germ cell-free system to yield an antiviral product with the properties of mouse interferon. About 700 units per  $\mu$ g added RNA were made. Similar results have also been described for RNA extracted from human cells in cell-free extracts from mouse ascites cells and rabbit reticulocytes (Pestka *et al.*, *Proc. natn. Acad. Sci., U.S.A.*, **72**, 3898-3901; 1975; Reynolds *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 4881-4885; 1975).

In view of these findings it is naturally to be expected that if such RNA preparations were to be injected into frog oocytes then translation of the exogenous mRNA would occur. That this is indeed the case has been demonstrated by Reynolds *et al.*, (*op. cit.*) who have achieved interferon titres of 100,000 units in homogenised oocytes after a 24 h incubation. Moreover, when  $^3$ H-leucine was included in the incubation and the product partially purified, using either immunoprecipitation with anti-interferon antibody or by affinity chromatography on a concanavalin A-agarose column, then subsequent electrophoretic analysis on polyacrylamide gels yielded a distinct peak of radioactivity with mobility corresponding to a molecular weight of 25,000 (this is about the expected value for human interferon). When RNA from cells not induced to make interferon was injected into oocytes very much less radioactivity was found in this peak. These results are striking when one considers that the injected RNA was the total mRNA fraction (containing poly(A)) from the induced cells and that the messenger for interferon could only comprise a very small proportion of this.

It seems likely that interferon is the first biologically active eukaryotic protein to be synthesised in a cell-free system dependent on added mRNA. But where does this get us? First it

## Interferon translated

from David Metz

INTERFERON is the antiviral protein synthesised by animal cells following infection with viruses. Since it is a cellular protein one would naturally suppose there to exist a corresponding mRNA species which could be translated in a cell-free protein synthesising system to yield, one would hope, the biologically active antiviral molecule. Three recent papers confirm this expectation.

The demonstration of the translation *in vitro* of interferon mRNA is made possible by the remarkably high biological activity of interferon. Although it has not yet been purified to unequivocal homogeneity, it is clear that the specific activity of interferon

must be at least  $10^9$  units of biological activity per mg protein. Thus the synthesis of exceedingly small amounts, by weight, of the active macromolecule may still be detectable by bioassay in which the reduction of virus yield is determined. A second convenient property of interferon is that of species specificity. Thus, for example, mouse interferon is active on mouse cells and not on human cells. Consequently if mRNA for human interferon is translated in an *in vitro* system prepared from mouse cells, one is able to verify that the antiviral activity synthesised is coded by the exogenous human mRNA and not by any endogenous murine messenger.



should enable the synthesis of interferon and its regulation to be studied in the intact cell since the mRNA as well as its product may be quantitated. Furthermore, *in vitro* synthesis of interferon should permit one to approach the question of the role of carbohydrate substitutions in interferon activity. Interferon has long been known to be a glycoprotein but whether the glycosylation is essential for activity remains unclear (if it is, then it must be occurring in the *in vitro* systems.)

Second, the apparent efficiency of the oocyte in translating interferon mRNA may assist in the determination of the primary structure of the protein, especially if the messenger can be at all purified from the other cellular mRNAs. The acquisition of sufficient pure interferon for amino acid sequencing by conventional techniques would be a formidable undertaking (though it is being attempted; Anfinsen *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3139-3142; 1974) since the very high specific activity implies the need for

very great volumes of comparatively low titre starting material. The ability of the oocyte to synthesise interferon highly labelled in various amino acids may well expedite the task of determining the primary structure. If this were known then chemical synthesis of the molecule could be attempted. If this in turn was achieved then it might, because of the very high specific activity, prove to be a more economic way of preparing the quantities of interferon required for clinical trials than the cell cultures now used. □

## The A, B, C viruses

from Arie J. Zuckerman

VIRAL hepatitis now constitutes the main hazard of transfusion of blood and certain blood products. It was estimated in 1972, for example, that transfusion-associated hepatitis caused more than 30,000 cases of overt hepatitis and 1,500 to 3,000 deaths each year in the United States (*US Department of Health, Education and Welfare*, 1972). Since there are many sub-clinical cases of viral hepatitis the actual incidence in the USA has been estimated as high as 150,000 cases annually.

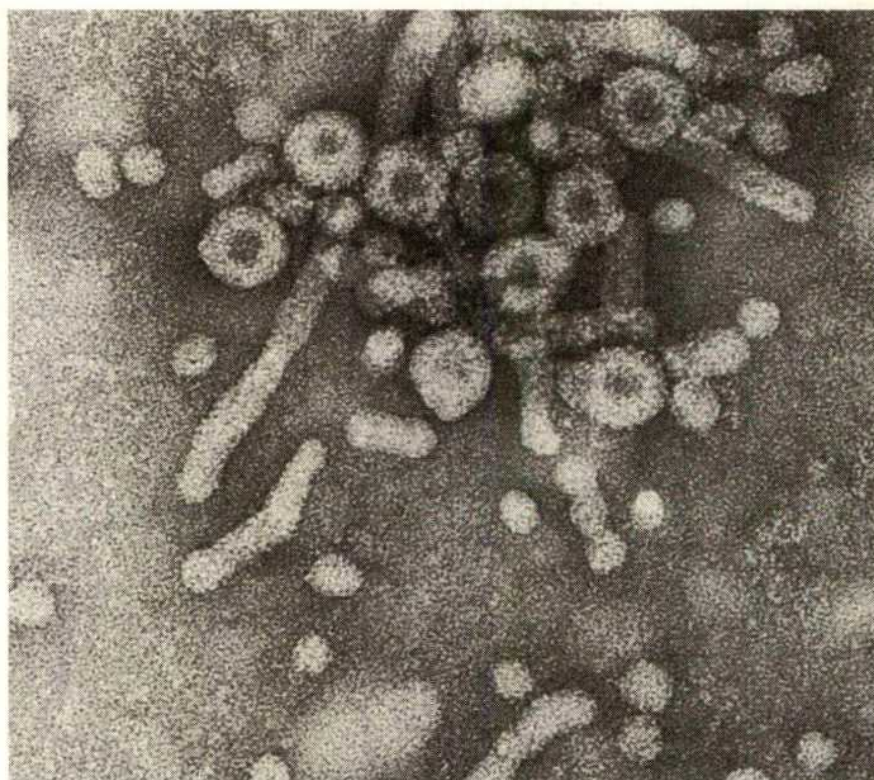
The existence of two types of viral hepatitis in man, infectious or epidemic hepatitis (hepatitis A) and serum hepatitis or homologous serum jaundice (hepatitis B) has been recognised for many years, and there is substantial evidence that these two types of infection are caused by antigenically distinct viruses. But it was difficult to distinguish clearly between these forms of hepatitis in the absence of specific laboratory tests. Differences were based principally on epidemiological observations and transmission experiments to human volunteers, faecal-oral transmission and an incubation period of about one month in the case of hepatitis A, and essentially tissue penetration and a long incubation period of up to six months in the case of hepatitis B.

The discovery of hepatitis B surface antigen (Fig. 1) and its association with infection has led to the development of a bewildering array of tests for screening blood donors and blood products. It was soon firmly established that blood containing this antigen was associated with a high risk of hepatitis and that such blood should not be used for transfusion. It rapidly became apparent, however, that the elimination of antigen-positive blood reduced post-transfusion hepatitis type B by about only 30% in some areas (*WHO Techn. Rep. Series*, No. 512, 1973). Although

the more recent introduction of sensitive methods of detection such as radioimmunoassay reduced further the incidence of post-transfusion hepatitis, cases of hepatitis B and non-B infections continue to occur (Koretz *et al.*, *Lancet*, **2**, 694; 1973). These remaining infections were generally ascribed to still insufficiently sensitive assay techniques for hepatitis B (Hollinger *et al.*, *New Engl. J. Med.*, **289**, 385; 1973 and *ibid.*, **290**, 1104; 1974) and to hepatitis A, which can also be transmitted by blood.

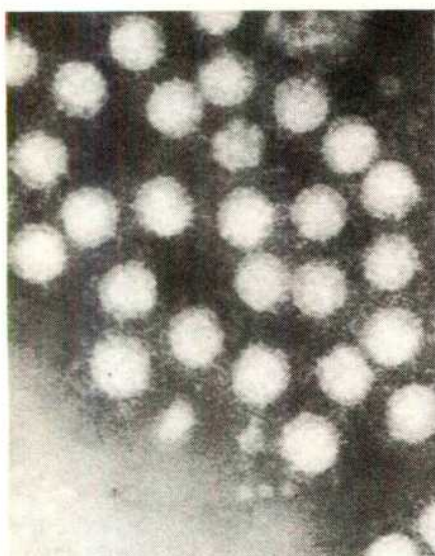
The identification by immune elec-

tron microscopy of virus-like particles (Fig. 2) in faecal extracts of patients with hepatitis A (reviewed in *Nature*, **252**, 193; 1974) and in experimentally infected marmosets and chimpanzees, provided an antigen which could be used for serological tests for hepatitis A antibodies and thus for evidence of infection. Immune electron microscopy has been used widely but immune adherence haemagglutination, complement fixation and radioimmunoassay techniques have been developed and are being applied on a limited scale. Sera from patients with hepatitis B-negative post-transfusion hepatitis in the United States were recently examined for evidence of infection with hepatitis A virus, and although relatively few patients have been tested so far sero-



**Fig. 1** The morphology of hepatitis B antigens showing three distinct entities, small 22 nm spherical particles, tubular forms of varying length and a large 42 nm double-shelled spherical particle. Original magnification  $\times 252,000$ . (Reproduced with permission from *Human Viral Hepatitis*, North Holland and American Elsevier, 1975.)





**Fig. 2** Virus-like particles measuring 27 nm in diameter found in faecal extract from a chimpanzee infected with human hepatitis A. Original magnification  $\times 275,000$ . (Electron micrograph from a series by A. Thornton, A. J. Zuckerman and J. D. Almeida.)

conversion to hepatitis A was not found in any of these patients (Feinstone *et al.*, *New Engl. J. Med.*, **292**, 767; 1975). A new term was coined; non-A: non-B hepatitis.

Feinstone and his colleagues were unable to implicate infection with cytomegalovirus or Epstein-Barr virus, which are known to induce liver damage as part of the generalised infection caused by these herpes viruses. Recently, Alter and his associates (*Am. J. med. Sci.*, **270**, 329; 1975) pointed out that in the United States when blood obtained from volunteer donors is pretested by radioimmunoassay for hepatitis B surface antigen approximately 90% of the remaining cases of post-transfusion hepatitis are serologically unrelated either to hepatitis A or hepatitis B viruses. It was considered, therefore, that a previously unrecognised human hepatitis virus may exist. This agent may be hepatitis virus C, a term introduced by Prince and his colleagues (*Lancet*, **2**, 241; 1974). They noted that an agent other than hepatitis B was the cause of 71% of 51 cases of post-transfusion hepatitis identified during a prospective serological study of 204 patients in New York. The incubation period was relatively long and the clinical and epidemiological features of the infection were not consistent with hepatitis A.

It is thus evident from these studies that a hepatitis virus C may indeed exist, although the precise criteria which would be virologically acceptable for a new infective agent have not yet been satisfied, despite the application of a battery of modern virological techniques. □

## Wigner cusps in nuclear reactions

from P. E. Hodgson

WHEN a proton is incident on a nucleus, many different reactions can take place. It may be elastically or inelastically scattered, it may be captured to form a compound nucleus which subsequently emits neutrons, protons or other particles, it may undergo (p,n) charge exchange, it may pick up a neutron to form a deuteron, it may knock out an alpha particle and so on. The relative probabilities of these processes depend on how easily the particles can get in and out of the nucleus (barrier penetration factors) and on the structure of the nucleus. The penetration factors depend on energy, and as the energy of the incident particle increases more and more reactions become possible.

At low energies the compound nucleus processes dominate, and then the cross section for all the non-elastic reactions depends on the penetration factor for the incoming particle, and this increases smoothly with energy.

As this energy increases this cross section has to be shared out among more and more of the reaction processes. The situation is rather like steadily increasing the flow of water into a closed tank, and at the same time making more and more holes of different sizes in the side of the tank. If we then concentrate our attention on the water coming out of one particular hole, we still see that at first it increases because of the increasing flow of liquid into the tank, and then decreases because more and more of the water is flowing out of all the other holes.

If we look very carefully, we may notice another effect: the flow of water out of our one hole falls slightly just after another hole has been made. This is because at any one time the total flow out must be the same as the total flow in, since the tank is closed and water is incompressible.

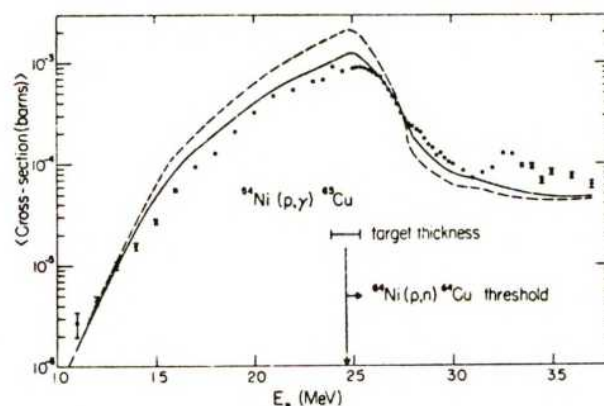
A very similar effect in nuclear reactions was predicted by Wigner in 1968 (*Phys. Rev.*, **73**, 1002). Each reaction process has a threshold energy: if the incident particle is below this energy the reaction cannot take place, but as soon as it is exceeded the cross section increases rapidly from zero. (This is the counterpart to punching another hole in the tank.) But since the total flux is constant, this must produce a small reduction in the cross section for all the other processes, and Wigner calculated the shape of the resulting cusps in the cross section as a function of incident energy.

It is interesting to try to observe these cusps as they would provide a delicate test of nuclear reaction theory but they are not easy to see. Usually there are so many hundreds or thousands of reactions taking place at the same time that the opening of another reaction channel, as it is called, does not make any perceptible difference.

The best conditions for observing Wigner cusps occur in the reactions of light nuclei, for in this case the level density is smaller and hence there are fewer channels. The effects of opening another channel are then more prominent, and indeed several cusps have been observed in light nucleus reactions, associated with the crossing of thresholds in neutron-induced reactions.

It is also interesting to look for Wigner cusps in proton reactions on heavier nuclei, and a remarkable example has recently been found in the  $^{63}\text{Ni}(p,\gamma)^{64}\text{Cu}$  reaction by Mann and his colleagues at the California Institute of Technology (*Phys. Lett.*, **58B**, 420; 1975).

This reaction was chosen because even though it is on quite a heavy nucleus it provides especially favourable conditions for the observation of



Excitation function for the  $^{63}\text{Ni}(p,\gamma)^{64}\text{Cu}$  reaction showing the Wigner cusps due to the crossing of the neutron threshold at 2.46 MeV. The curves show the results of Hauser-Feshbach statistical model calculations with (full) and without (dashed) the width fluctuation correction.

a Wigner cusp. These are that the new channel opens quickly, absorbing an appreciable fraction of the total flux, and that there are very few channels open already to share the resulting decrease in flux. These conditions are very well satisfied for the  $(p,\gamma)$  channel on  $^{64}\text{Ni}$  at the opening of the neutron threshold. The lowest excited states of  $^{64}\text{Ni}$  are at relatively high energies and this, together with the low energy of the neutron threshold (opening of the  $(p,n)$  channel), reduces the contribution of the inelastic scattering and compound elastic processes to the total reaction cross section. A possible competing reaction, alpha particle emission, is strongly inhibited by the Coulomb barrier, so that the total reaction cross section below the  $(p,n)$  threshold is mainly composed of the  $(p,\gamma)$  channels. Another favourable feature is the high density of low-lying states in  $^{64}\text{Cu}$ , the final nucleus in the  $(p,n)$  reaction, which ensures that the total  $(p,n)$  cross section increases very rapidly, producing a strong depletion in the  $(p,\gamma)$  cross section.

The measurements of the  $(p,\gamma)$  cross section were made from 1.1 to 3.7 MeV in 100 keV steps, and the yields for the reaction to the first three excited states of  $^{64}\text{Cu}$  were summed to give the results shown in the figure. It is notable that the cross section increases steadily from 1 to 2.5 MeV and then drops dramatically by a factor of 10 over the next 0.5 MeV as a result of the opening of the  $(p,n)$  thresholds. In this case the Wigner cusp is not a tiny perturbation but a major feature of the cross section. Many neutron thresholds are passed above 2.5 MeV, and these all contribute to the drop in the  $(p,\gamma)$  cross section.

A small resonance is visible in the  $(p,\gamma)$  cross section at about 3.27 MeV. This is attributed to the state in  $^{64}\text{Cu}$  that is the analogue of the first excited state of  $^{63}\text{Ni}$ .

At such low energies the reaction is dominated by the compound nucleus process; the incident proton is captured by the  $^{64}\text{Ni}$  nucleus to form a compound nucleus  $^{65}\text{Cu}$  and after a long time on the nuclear scale it emits one or more gamma rays until it returns to the ground state. The cross sections for such processes can be calculated by the Hauser-Feshbach statistical theory, using barrier penetration factors calculated from the appropriate optical model potentials. Since the number of open channels is small, this reaction is a severe test of the theory.

The results of Hauser-Feshbach calculations are shown by the dashed curve in the figure, and are in qualitative agreement with the measurements. If account is also taken of the correlations between the partial widths in the

entrance and exit channels by including the width fluctuation correction the improved results shown by the full line are obtained.

This comparison shows that the Hauser-Feshbach theory is well able to account for the Wigner cusps, and that it is important to include the width fluctuation correction.  $\square$

## Disappearing habitats

from Peter D. Moore

It is believed that the Romans were responsible for the construction of an extensive series of draining channels in the low-lying coastal area between Newport and Cardiff in South Wales, commonly known as the 'Monmouthshire levels'. The value of this land, mainly for pasture, is maintained by a careful control of the water table, which has long been effected by these drainage ditches, locally termed 'reens'. The reens have an additional value in that they represent a diverse series of habitats for aquatic plants and animals and serve as an extensive refuge for a large number of plant and animal species.

Recently this area has come under threat from a number of quarters and an ecological survey of the area and the various interrelationships between such physical factors as water table and plant and animal communities was urgently required. Just such a survey has been conducted recently by P. M. Wade of the University of Wales Institute for Science and Technology, Cardiff, and some of the conclusions were presented during a recent meeting of the British Ecological Society in Cardiff.

The floristic diversity of the reens has been confirmed by Wade's study, in which he recorded over 400 plant species associated with the waterways and their adjacent hedges and meadows. He feels, however, that recent land use changes in the area are combining to reduce this diversity.

The drainage channels are arranged in a rectangular grid system, where subsidiary reens coalesce into main reens which ultimately discharge their load into the Bristol Channel through simple, one-way sluice gates. These gates are not always perfectly efficient and there is an occasional movement of estuarine water back into the reens, which serves to diversify yet further the habitats within these channels. The fields which are bordered by the reens are drained by small furrows on their surface, from which run-off water reaches the reens.

Recent land-use changes have included the installation of sub-surface drainage pipes which have resulted in a more efficient water-ridding system and a consequential improvement of the meadows for pasture. This has meant that field size can be increased by reducing the number of reens, either by allowing them to silt up or by actively filling them. It has also allowed an increase in stock density to take advantage of the improved pasture yield.

Historically, the reens have served the subsidiary function of providing water for the resident cattle. This practice has also brought its problems, because the trampling of stock around the ditches has damaged banks and increased the rate of silting. An increased stock density, of course, makes this an even more serious problem. An answer has been found in the provision of water troughs situated in the centres of fields which attract animals away from the ditches.

At this stage a farmer is bound to ask the question whether he needs the reens any more. Their maintenance is expensive; at one time they were dug out by hand, but now excavation can be carried out mechanically. This, however, has involved the removal of most of the accompanying hedgerows which themselves provided food and shelter for wildlife. The growth of emergent aquatic plants can be controlled by spraying herbicides and this reduces the need for frequent ditch clearance, but spraying is itself an expensive process particularly because of the labour involved. So, since the reens are no longer useful as a water resource for livestock, it seems advantageous to lower the water levels within them or even to empty them completely. The use of mechanical pumps to replace the earlier crude sluice system has now made this possible.

Inevitably these changes have become more widespread until the traditional management of the reens has practically disappeared and subsequently the character and the biotic richness of the habitats which they represented is also vanishing. At this point one can predict that naturalists and wildlife conservationists will begin to muster and cry out for the retention of the old system in at least a portion of the area occupied by these low-flying levels. It is not difficult to summon up sympathy for such views, especially since they emanate from a part of the country where man's recent activities have reduced biological diversity to an extraordinarily depauperate level. The remaining fragments must assume a particularly high status in the eyes of those members of the local populace who appreciate such things. One cannot help reflecting, however, upon the



innate conservatism of mankind and one wonders whether the Romans were faced by a similarly militant Celtic environmental lobby when first they dug the ditches! □

## The EISCAT project

from G. N. Taylor

THE recent announcement that the UK Science Research Council is joining EISCAT draws attention to some significant current developments in upper atmospheric research. EISCAT stands for the European Incoherent Scatter Organisation, an association of research organisations from six countries which has been set up to build and operate a large new radar system in the auroral region of Northern Scandinavia. In this case the capital and running costs, and overall control of the project, are being shared between Research Councils or similar bodies in Britain, France, Finland, West Germany, Norway and Sweden. After preparatory discussions and technical studies lasting several years it has been decided to base the main technical centre at Tromsø in Norway, and the administrative headquarters at Kiruna in Sweden. The organisation will be governed by a Council made up of representatives from all the parent bodies, and Professor Tor Hagfors from the Technical University of Trondheim has been appointed as the first Director of the facility.

The incoherent scatter technique originated from a proposal by W. E. Gordon in 1958, for building a very large radar system in order to detect weak Thomson scattering from thermal electrons in the ionosphere. Originally conceived as a way of measuring electron density and temperature in the upper ionosphere from the ground, the method has developed into one of the leading diagnostic tools not only for the upper atmospheric plasma but also for thermonuclear plasmas. From experimental work done in the 1960s, it was soon discovered that both electron and positive ion temperatures could be measured independently, and later that plasma drift speeds and fluxes of super-thermal electrons could also be determined. From these direct measurements, which are made as functions of height and time, many other physical parameters can be derived indirectly: among the most important are the electrostatic field strength, the temperature and wind speed of the neutral air, and energy fluxes. In collaboration with other ground-based techniques, or with rocket or satellite-borne instruments, incoherent scatter can give remarkably

complete information about physical processes and conditions in the upper atmosphere. Incoherent scatter does not however supersede satellite experiments, even though some of the parameters measured are the same. For complete understanding, the upper atmosphere must be seen as a three-dimensional time-varying medium: the radar works in height and time, whereas most satellite measurements are expressed as functions of latitude and longitude.

Incoherent scatter radars are complicated and expensive, requiring very high power transmitters, large antennae and sophisticated data processing equipment. Until now, no more than seven stations world-wide have ever been operating at the same time, and most of these have been at middle latitudes. The EISCAT facility will be only the second station in either of the auroral zones, which are the key regions for observing interactions between the ionosphere, the magnetosphere, the neutral atmosphere and the interplanetary plasma, and where many important and poorly understood phenomena occur. The radar should come into operation in 1978 or 1979, and will then be operated intensively for at least a decade, with its time shared between a routine synoptic programme and special experiments by scientists from the participating countries. In Britain, research groups from a number of Universities and from the SRC's Appleton Laboratory are planning to use the facility. The scientific scope for European workers will be enhanced by the proximity of two rocket launching sites, and of many other ground-based instruments, at well established upper atmospheric observatories in adjacent areas

of each of the three Scandinavian countries. A wide variety of other data will thus be available to complement and enhance EISCAT observations.

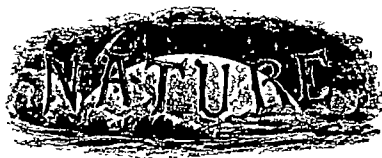
In contrast to most of the earlier incoherent scatter sites, the EISCAT system will be designed solely for upper atmospheric research. It will be unusual in having two radars on widely different frequencies. One in the VHF band (at 224 MHz) will be a conventional monostatic type, designed to achieve the maximum sensitivity, and to explore the highest and lowest regions. The other will operate in the UHF band (at 933 MHz), and will be a multistatic radar of the type pioneered in France, and also used in Britain, capable of determining the true vector plasma drift velocity. The transmitters for both radars will be at Tromsø: the VHF receiver and one UHF receiver will be co-sited with the transmitters, while remote UHF receiving stations will be built at Kiruna and at Sodankylä, in Finland, respectively 200 and 400 km distant from Tromsø. Both radars will be designed with very flexible modes of transmission modulation, which should allow for a wide variety of experiments to be conducted. Comprehensive on-line and off-line data processing equipment will also be provided to obtain the maximum possible rates of useful information, and to monitor the progress of observations in real time. These features, together with the uniquely favourable geographical position of the site, should allow European scientists to make significant new contributions to an important field of geophysics. □

## Bacterial chemotaxis and methionine

from John S. Parkinson

NEARLY a century ago, Engelmann, Pfeffer and others showed that bacteria are attracted or repelled by various chemicals. Today studies on chemotactic behaviour in *Escherichia coli* and other bacteria have begun to reveal molecular mechanisms of sensory transduction and motility in micro-organisms. The central machinery of chemotaxis is still, however, very much a mystery. Several recent reports suggest that the amino acid methionine may provide the biochemical key to understanding chemotactic behaviour in *E. coli*.

The methionine requirement for chemotaxis is a specific and continuous one. *E. coli* mutants that are unable to synthesise methionine become non-chemotactic when deprived of exogenous methionine and chemotactic ability returns upon restoration of this amino



### A hundred years ago

THE first general meeting of the Mineralogical Society of Great Britain and Ireland is held to-day at the Scientific Club, Saville Row, at 12 o'clock (noon), when the chair will be taken by Mr. H. C. Sorby, F.R.S. The first ordinary meeting will be held at the same place and time to-morrow, when a paper will be read on the Scottish Rhombohedral Carbonates, by Prof. M. Forster Heddle, M.D.

from *Nature*, 13, February 3, 274; 1876

acid. Since no other amino acids produce such an effect, it cannot be related simply to effects on protein synthesis. In fact, ongoing protein synthesis is not needed for chemotaxis once the machinery has been assembled.

The behavioural manifestation of methionine deprivation is a change in the organism's swimming pattern. Normal swimming in *E. coli* resembles a random walk in three dimensions with occasional turning or tumbling movements serving to reorient the direction of forward motion. During chemotaxis the cell's tumbling machinery is modulated by chemoreceptors that detect changes in attractant or repellent concentration. Whenever the organism happens to swim toward an attractant or away from a repellent, tumbling is suppressed, which causes net movement in the appropriate direction. Methionine-starved cells are non-chemotactic because they are no longer capable of tumbling. Methionine therefore seems to have a major role in the production or regulation of tumbling movements, events central to the chemotactic process.

Springer *et al.* (*Proc natn Acad. Sci U S A*, **72**, 4640-4644; 1975) have tried to determine, by examining the effects of methionine deprivation on *E. coli* mutants with very high tumbling rates (tumbly mutants), whether methionine was directly involved in the tumbling process. They found that methionine starvation caused several of the mutants to lose tumbling ability, and that the time required for complete loss of tumbling was related to the chemotactic ability of the strains. Tumbly strains with poor chemotactic responses continued tumbling for several hours after removal of methionine from the medium. One mutant, in fact, never lost the ability to tumble.

Springer *et al.* also showed that exhaustion of the organism's internal reserve of methionine available for tumbling was accelerated by incubating the bacteria in attractant compounds during methionine starvation. Although unchanging attractant concentrations do not ordinarily modify tumbling behaviour, they evidently elicit increased consumption of methionine by the chemotaxis machinery.

Until more is known about the physiological defects of tumbly mutants, it is not possible, on the strength of present evidence, to conclude that methionine is directly used in generating tumbles. The data of Springer *et al.* and results of Aswad and Koshland on *Salmonella typhimurium* (*J. Bact.*, **119**, 640-645; 1974) are perhaps best explained by postulating an indirect role for methionine in the tumbling process. For example, methionine might be consumed in destroy-

ing tumble-inhibiting signals produced by the chemoreceptors. In the absence of methionine these signals would build up (faster in the presence of attractants) until tumbling was suppressed. Tumbly mutants might be less sensitive to such signals, necessitating higher signal levels (hence longer starvation times) to turn off the tumbling machinery in these strains.

A more direct way of investigating the role of methionine in chemotaxis is to determine the chemical fate of exogenously supplied methionine. Earlier work by Armstrong and by Aswad and Koshland had suggested that a compound derived from methionine called SAM (S-adenosylmethionine) was involved in chemotaxis. Since SAM is a donor of methyl groups in many biochemical reactions in the cell, Kort, Goy, Larsen and Adler (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 3939-3943; 1975) reasoned that perhaps the methyl group of methionine is transferred by way of SAM to one or more components of the chemotaxis machinery. To show this, they looked for transfer of radioactive label from the methyl group in methionine to proteins of the cytoplasmic membrane in the absence of *de novo* protein synthesis. Their search focused on membrane proteins because most of the known chemotaxis machinery is intimately associated with the cell's inner membrane.

Kort *et al.* were able to detect a membrane protein that is extensively methylated by methionine. Chemotactic stimuli increase both the rate of methionine utilisation and the amount of label transferred to the membrane protein. Also, the methyl groups on the membrane protein seem to turn over, perhaps reflecting the continuous requirement for methionine in chemotaxis. Chemotaxis mutants, including tumbly and non-tumbling strains, often had very different labelling patterns compared with the wild type. Most of the mutants (defining several different genes) had significantly less activity and correction of these mutations by reversion also resulted in normal methylation levels.

So the methylation reaction seems to be involved in chemotaxis and might conceivably be the primary site of action of methionine in the chemotactic process. But Kort *et al.* found some chemotaxis mutants (in the same genes as those mentioned above) which had normal or greater than normal methylation levels and yet were completely non-chemotactic. Detailed information about methylation and demethylation rates in these mutants should clarify the presently confused relationship between methylation and chemotactic ability.

Even if the role of methionine in bacterial chemotaxis proves to be a minor one, which seems unlikely, it still offers a promising new approach to learning more about the biochemistry of the tumbling process. Future studies will undoubtedly attempt to identify the gene(s) encoding the methyl-accepting membrane protein and to determine their role in chemotaxis through a combination of genetic and biochemical methods. When this has been accomplished, the significance of methionine to chemotaxis will be considerably clearer. □

## Heated discussions on Sun and weather

from John Gribbin

1976 seems destined to be the year of the Solar-Terrestrial Relationship conference. International jamborees on the topic are planned later in the year for Frascati (under the auspices of the European Space Agency) and Boulder, Colorado (organised by the American Geophysical Union); a more limited view of these relationships, within the confines of their relevance to meteorology and climate, was the theme of the January 21 meeting of the Royal Meteorological Society, where although the staunch believers in direct solar effects on the weather received a rough ride from some meteorologists there was a firm but reluctant undertone of belief even among the critics that there is evidence of some effect of solar activity on weather.

THE highlight of the meeting was a confrontation between J. W. King (Appleton Laboratory), a firm believer in solar cycle influences on the weather, and B. J. Mason, the Director General of the UK Meteorological Office, the most senior 'establishment' opponent of these ideas. King presented a breathtaking array of data in the form of graphs, charts and correlations between meteorological parameters such as the height of the 500 mbar level and solar parameters, especially the 22 yr cycle which, in magnetic terms, is a more fundamental feature than the superficial 11 yr cycle derived simply by counting sunspots. Among these correlations—in phase as well as in magnitude—most of the figures quoted by King suggested a probability of at least 99% that the relationships are genuine, and he tended to dismiss probabilities of around 95% as rather poor.



Mason responded at length to King's points, complaining that he had presented too many different correlations for the audience to grasp at one sitting, pointing out that they are all *post hoc* deductions and that sunspot cycle fanatics have now had "a hundred years of failure" in attempting to predict weather changes. He stated once again his unwillingness to accept such statistical evidence in the absence of a plausible physical mechanism behind it.

Asked how he had selected the data presented, King replied that they are typical of results obtained by comparing solar activity with published meteorological data, and that in round terms the Appleton team find in 95% of all the published data they have examined a correlation which could arise only about 2% of the time by chance. Mason objected that this meant the data were not new, but when pressed by King to say whether meteorologists should throw out the evidence replied "No", agreeing that solar effects must influence the high atmosphere, and asking that the theorists concentrate on explaining those effects before looking "downstairs" in the troposphere.

Two members of the Met. Office then described different studies in which rigorous statistical analysis had failed to remove every trace of a solar influence from their data. B. N. Parker surveyed links between the planetary mean geomagnetic index and surface pressure anomalies on a monthly timescale over the Northern Hemisphere, and C. K. Folland looked at relationships between sunspot and other quasi-cyclic fluctuations and circulation over the British Isles. The evidence seemed to show significant but not dominant solar influences in some stretches of data at least—not a good enough relationship for forecasting purposes, but suggestive for any theorist studying interactions between the Sun and the Earth. Again, the 22 yr cycle reared its head, and meteorologists took some persuading that this is indeed the more basic solar cycle, not just a harmonic of the 11 yr sunspot number cycle. All this, unfortunately, kept the emphasis of the meeting well away from the short term effects of specific solar events on the atmosphere of the Earth, which surely provide a better handle to grasp the physics of what is going on than any number crunching of cycles, quasi-cycles and pseudo-cycles.

It was left to D. M. Willis (Appleton Laboratory) to summarise possible mechanisms. It might have come as a surprise to meteorologists sceptical of some of King's statistics to learn how much room there is to doubt astrophysical ideas about the Sun and the constancy of the solar parameter. Among many speculations aired before, Willis

rightly emphasised the newer ideas of the influence of solar proton events on the atmosphere. This work is closely related to the study by Reid *et al.* (*Nature*, 259, 177; 1976) of faunal extinctions at times of magnetic reversals, and suggests that solar cycle influences on the weather may be the accumulation of solar proton effects on the ozone layer. It is also clear, however, that the state of the art in measuring the solar parameter is not yet good enough to rule out the possibility of a change by  $\pm 1\%$  over the solar cycle, changing mean global surface temperatures by  $\pm 1$  K.

The overall flavour of the meeting seems clear. Solar-Terrestrial relationships affecting the weather are real, but not dominant, and are not well enough understood for their use in Met Office forecasts as yet. But they may provide clues of great value to astronomers investigating the nature of the Sun and solar wind. This explains, perhaps, the apparent rift between meteorologists and space physicists; in fact, they are essentially in agreement on the reality of the link, but view it from very different positions. □

## Let booming sands boom

from Peter J. Smith

HARDLY surprisingly in view of their weird effects, sounding sands are incorporated into folklore and legends going back at least 1,500 years. Not that our ancestors were always listening to the same sounds. The most common of the musical sediments is probably squeaking (otherwise known as singing, barking or whistling) sand which produces a high frequency note in the range 500–2,500 Hz. But there is also screeching sand which emits an even higher frequency note ( $>2,500$  Hz) reminiscent of that produced by rubbing a finger around the top of a wine glass; and at the other end of the scale, low frequency booming sand can give rise to a sound like thunder which under ideal conditions can be heard up to 10 km from its source. The essential condition for the production of sound is either natural or forced movement; still sand is inevitably silent sand.

A recent paper by Criswell *et al.* (*J. geophys. Res.*, 80, 4963; 1975) investigates booming sand, which generates not only acoustic but also seismic waves. Both emissions are produced by natural slumping of the sand,

although they may also be produced manually in short bursts by digging in the sand, by forcing it downhill or even by walking on it. The natural slumping of an area of many square metres of sand may persist up to 15 min, giving rise to a 'roar', or on occasions a 'hum', rather like the sound of a low-flying propeller aircraft. But whatever the sound, it is always accompanied by ground vibrations which can be felt through the feet of anyone standing on or near a booming dune and through the fingers of someone generating artificial booming by digging in the sand. One observer has compared such vibrations to a mild electric shock from household current (50–60 Hz).

Though not particularly common, booming dunes have been reported at more than 30 sites in all continents except Antarctica and, curiously, Australia. The sound frequencies at some of these sites have been estimated before by reference to pitch pipes; but Criswell *et al.* have now carried out the first simultaneous measurement of acoustic and seismic spectra from a booming dune (Sand Mountain in Nevada) using, respectively, air microphones and geophones. The emissions were generated artificially by digging holes in the sand with a flat-bladed shovel.

Not entirely unexpectedly, the scientific aspects of booming dunes turn out to be rather dull and hardly a match for the subterranean ghosts, the shoeing of horses in underground caverns and the clanging of bells in buried monasteries to which sand sounds have been attributed in the past. The most "significant finding" is that the frequency spectrum of a short ( $<2$  s) forced booming event comprises sharp peaks largely in the range 50–80 Hz for both acoustic and seismic emissions. These results throw little light on the mechanism by which the vibrations are generated, which remains unclear; nor do they do much to justify the original aim of the work, which was to see whether a similar mechanism on the Moon could account for surface moonquakes.

Criswell and his colleagues do obtain one interesting result, however. A comparison of an acoustic amplitude trace from a Sand Mountain note with the trace of an 88-Hz organ tone from the opening stanza of Bach's C-Minor Passacaglia and Fugue shows that sand and expertly crafted organ pipes produce notes of comparable purity.

Perhaps Criswell and others should take the hint from Walt Whitman who, tiring of the learned astronomer's proofs, figures and charts, "wander'd off . . . in the mystical moist night-air, and from time to time, look'd up . . . at the stars." □

# articles

## Receptor-binding region of insulin

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*X-ray analysis, circular dichroism, receptor binding and biological potencies of chemically modified insulins suggest that the conformation of the insulin molecule is critical to the formation of both the zinc insulin hexamer and the insulin-receptor complex. Results are consistent with an insulin receptor-binding region including many of the hydrophobic residues important to dimerisation in addition to more polar surface residues. There is a further possibility of formation of an antiparallel sheet structure between the insulin and receptor molecules in the complex similar to that between monomers in the insulin dimer.*

THE use of synthetic insulin analogues in the treatment of diabetes may become vitally important in the near future. Progress in presently underdeveloped countries means that demand for insulin is likely to exceed supply and this makes the synthesis of analogues a medical priority.

An understanding of the nature of the receptor-binding region of a protein hormone, and especially as complex a molecule as insulin, is essential for the proper design of such synthetic analogues. Fortunately the elucidation of the molecular structure of porcine insulin by X-ray analysis has made possible a more rational approach to the definition of the receptor-binding region<sup>1,2</sup>. Studies of the biological activities and sequences of insulins from different animals strongly suggest that a largely invariant region on the surface of the insulin monomer is a good candidate for this receptor-binding region. The three-dimensional structure shows that this region might include both A chain residues—A1 Gly, A5 Gln, A19 Tyr and A21 Asn—and adjacent B chain residues—B24 Phe, B25 Phe, B26 Tyr, B12 Val and B16 Tyr (for reviews see refs 3 and 4). Their involvement in receptor binding must now be tested by studying the biological properties of insulins specifically modified at one of these residues.

Modification of insulin may change the biological activity either by reducing the affinity of the hormone for the receptor or by decreasing the ability of the complex, when formed, to elicit a biological response. Either or both these effects may be brought about by direct interference of the modifying group, by changes of charge distribution or by induced conformational changes in the hormone. Thus the position of the modifying

group and the conformational changes induced by it must be defined in detail; this has not been done in previous work. Further, measurements of receptor binding as well as biological activity are essential to test any hypothesis concerning the location of the receptor-binding region, and the effect of chemical modification<sup>5</sup>.

We have carried out parallel studies of high resolution X-ray diffraction, circular dichroism, receptor binding and biological potency in fat cells of native insulin and various chemically modified insulins. The studies have further clarified the factors leading to decreased potency and provide evidence as to the nature of the receptor-binding region.

### Chemical modification and X-ray analysis

We studied a series of insulins with modifying groups of increasing size attached to the  $\alpha$ -amino group of A1 glycine, one of the invariant residues in the putative receptor-binding region. Techniques of specific chemical modification, developed in our laboratories, were used to prepare A1-acetyl insulin<sup>6,7</sup>, A1-*t*-butoxycarbonyl insulin<sup>8</sup> (A1-Boc-insulin) (this has also been prepared by Levy<sup>9</sup>) and A1-2-dimethyl-3-formyl-L-thiazolidine-4-carbonyl insulin (A1-'thiazolidine' insulin)<sup>10</sup>. A1-Boc-insulin was in part provided by H.-J. Friesen, Aachen (Diplom. Arbeit, T. H. Aachen, 1975).

Rhombohedral crystals of bovine derivatives formed more easily than those of porcine derivatives. They crystallised at pH 5.8 as opposed to pH 6.2 for native porcine insulin, whereas large crystals of native bovine insulin were prepared only at pH 6.7. For this reason we have studied modified bovine insulins, and also carried out control experiments on native bovine insulin.

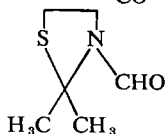
Crystals of the chemically modified and bovine insulins proved to be closely isomorphous with porcine insulin although there are small changes in cell dimensions as indicated in Table 1. X-ray photographs of the A1-'thiazolidine', A1-Boc and A1-acetyl-insulins resemble each other, but the extent of the intensity changes from native insulin increases in the order A1-acetyl < A1-Boc < A1-'thiazolidine' insulin.

Three-dimensional X-ray data for the isomorphous bovine, A1-Boc and A1-'thiazolidine' insulins were collected on a four-circle diffractometer to the resolutions given in Table 1. As the differences between the A1-acetyl and bovine insulins were small, but rather similar in nature to the other two derivatives, we decided that it was not useful to carry out a full high resolution analysis of this derivative.

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Table 1 Comparison of insulins

Insulin	Modifying group	Cell dimensions (Å)		Data resolution (Å)
		$a = b$	$c$	
Porcine	—	82.5	34.0	1.9
Bovine	—	82.5	33.8	2.3
A1-acetyl	CH <sub>3</sub> -CO-	82.5	33.8	—
A1- <i>t</i> -Boc	(CH <sub>3</sub> ) <sub>3</sub> C-O-CO-	82.5	33.5	3.0
A1-'thiazolidine'	-CO-	82.5	33.8	2.3

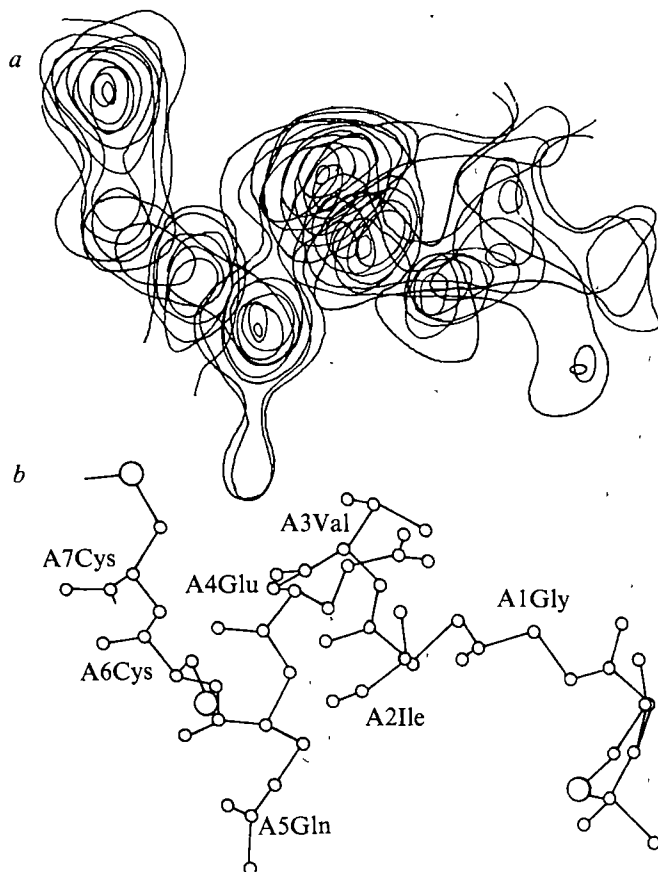


Structural differences between the insulins were investigated using a series of electron density maps computed with coefficients

$$m(nF_D - (n-1)F_P) \exp i\alpha_P$$

where  $F_P$ ,  $m$  and  $\alpha_P$  are the observed structure factor amplitudes, the figure of merit and the best phase for the porcine insulin crystals, and  $F_D$  is the observed structure factor amplitude of the derivative crystals. Values of  $m$ ,  $\alpha_P$  and  $F_P$  for porcine insulin crystals were provided by Professor Dorothy Hodgkin and her colleagues. Conventional difference maps, with coefficients  $(F_D - F_P)$  were used to indicate changes of electron density between the porcine and derivative crystals. But electron density maps calculated with  $n = 2$  were used in building models of the derivative structure as the map should give a reasonable approximation of the derivative crystal electron

Fig. 1 View of (a) electron density and (b) molecular structure for the A-chain helix and neighbouring residues of A1-'thiazolidine' insulin, the projection corresponds to a depth of 10 Å through the structure of the  $n = 2$  map obtained for the derivative (see text).



density and can be compared directly with a model if an optical device is used.

These electron density maps indicate that the bovine insulin dimer is closely similar to the porcine dimer, although there are small changes in the position of one zinc atom and indications of some further cation binding and of a small shift of the molecule in the unit cell. Negative peaks in the difference maps correspond to the sequence differences: A8 Thr and A10 Ile in porcine insulin are replaced by A8 Ala and A10 Val in bovine insulin and these changes are thought to be responsible for the slight differences in packing observed in the bovine and porcine structures. Similar differences of packing occur in the A1-Boc and A1-'thiazolidine' bovine insulins; in addition in both derivatives the region around A1 shows extensive conformational changes in both the molecules of the crystallographic dimer. The changes are qualitatively similar in the two derivatives but are greater in magnitude in A1-'thiazolidine' insulin.

Figure 1 shows the electron density and molecular structure close to A1 in molecule (2) of A1-'thiazolidine' insulin. The thiazolidine group is extended into the solvent region, apparently displacing water molecules, and comprises a distorted extension of the A-chain helix. The Boc group occupies a similar position in A1-Boc insulin. The electron density of the 'thiazolidine' and Boc insulins indicates a rotation of the helix A2-A5 around its axis in a clockwise sense when viewed from the N-terminus. The side chains of A2 Ile, A4 Glu and A5 Gln have been pushed away from the A1 position and the side chain of A19 Tyr has shifted about 1.5 Å from A2. A similar rotation and displacement of side chain groups occurs in the other molecule (1) of the dimer, but the modifying group appears to be closer to the helix axis and the shift of A5 side chain is less. There is also a very small displacement of the residues B24 to B28 away from the modifying groups at A1.

### Circular dichroism

Spectra of chemically modified insulins have been published<sup>7,12-14</sup> but interpretation is difficult since chemical modification usually affects quaternary structure<sup>15</sup>. We have shown elsewhere that changes in circular dichroism resulting from changes in insulin concentration and the zinc-insulin ratio can be accounted for by changes of environment of the chromophores on changing the equilibrium populations of monomers, dimers and zinc hexamers. We concluded that the structure of the protomer seen in the crystals of zinc insulin hexamers is largely retained by the molecule in solution. Thus chemical modification of insulin may affect the circular dichroism by changing the population of associated molecules, the overall tertiary structure of the modified insulin being essentially the same as the native molecule.

We have measured the circular dichroism spectra using a Cary 61CD spectrometer, in both the near and far ultraviolet in 0.025 M Tris-HCl buffer pH 7.79, for the A1-'thiazolidine' and A1-Boc insulins in similar concentration ranges and in the presence of zinc ions. Representative spectra are shown in Figs 2 and 3. The spectra of the derivatives at a given concentration correspond generally to the spectra of the native protein

at higher dilutions, indicating that these derivatives probably associate less strongly than native bovine insulin. A similar comparison of the spectra of the two derivatives suggests that A1-'thiazolidine' insulin associates less than the A1-Boc insulin. This decrease in association can be explained by the change of charge at A1 and small conformational changes induced in the B chain residues B24-B28 which are directly involved in dimerisation. We have already observed that such changes occur in the electron density map but it is probable that the differences between native and derivative structures are slightly greater in the isolated monomer, being decreased in the dimer when the  $\beta$ -sheet is formed. The need to readjust the structure of the modified insulins slightly on dimerisation would explain the decreased ability to dimerise, and account for the decreased dimerisation of A1-'thiazolidine' insulin compared with A1-Boc insulin, a difference which cannot be explained by changed charge or direct interference of the modifying group.

Apart from the shifted concentration and zinc dependence of the circular dichroism, the spectra are very similar, indicating that the derivative insulins also have structures in solution comparable with bovine insulin. There are, however, small differences in ellipticity in both near and far ultraviolet between bovine and derivative insulins at all states of association which may arise from the movement of the A19 tyrosine chromophore as observed in the crystal structure. The general structure of the derivative insulin molecules in solution, however, is probably very similar to that in the crystal.

### Biological potency

The potency has been assessed by incorporation of 3-<sup>3</sup>H-glucose into fat cell lipids<sup>16,17</sup>. The relative potency of A1-acetyl insulin has been determined as 35-40% (refs 7 and 18). The derivatives exhibit the same maximal activity (efficacy) as insulin with respect to lipogenesis from glucose as has been found for several modified insulins<sup>18</sup>. In the experiment shown in Fig. 4a, (left) A1-Boc insulin exhibited a relative potency of 15.6%. The mean of five such experiments was  $17.0 \pm 3.1$  (s.d.). Figure 4a (right) shows a similar experiment with A1-'thiazolidine'

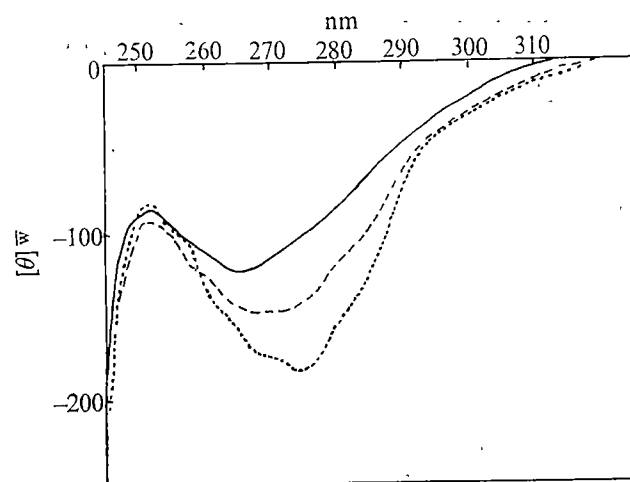


Fig. 2 Near ultraviolet circular dichroism spectra of zinc-free A1-t-Boc insulin at  $10^{-6}$  M (—),  $10^{-5}$  M (---) and  $10^{-4}$  M (···), showing the dependence of the spectrum on protein concentration. A similar concentration dependence is shown by bovine insulin.  $\bar{w}$ , Mean residue weight.

insulin and the mean of five experiments was  $12.8 \pm 1.6$  (s.d.). It is further seen from Fig 4a that A1-'thiazolidine', when added to native insulin in the concentration ratio 10:1, and A1-Boc insulin (ratio 5:1) had additive effects in submaximal doses. This is characteristic of a full agonist which is acting through the same mechanism and with the same rate-determining step as native insulin.

Receptor binding of the insulin derivatives was determined as their ability to inhibit binding of <sup>125</sup>I-insulin to fat cells as previously described<sup>16</sup>. Figure 4b shows that the relative binding affinities of A1-Boc insulin and A1-'thiazolidine' insulin are both similar to the relative potencies. This means that the ratio between the concentration which causes half-

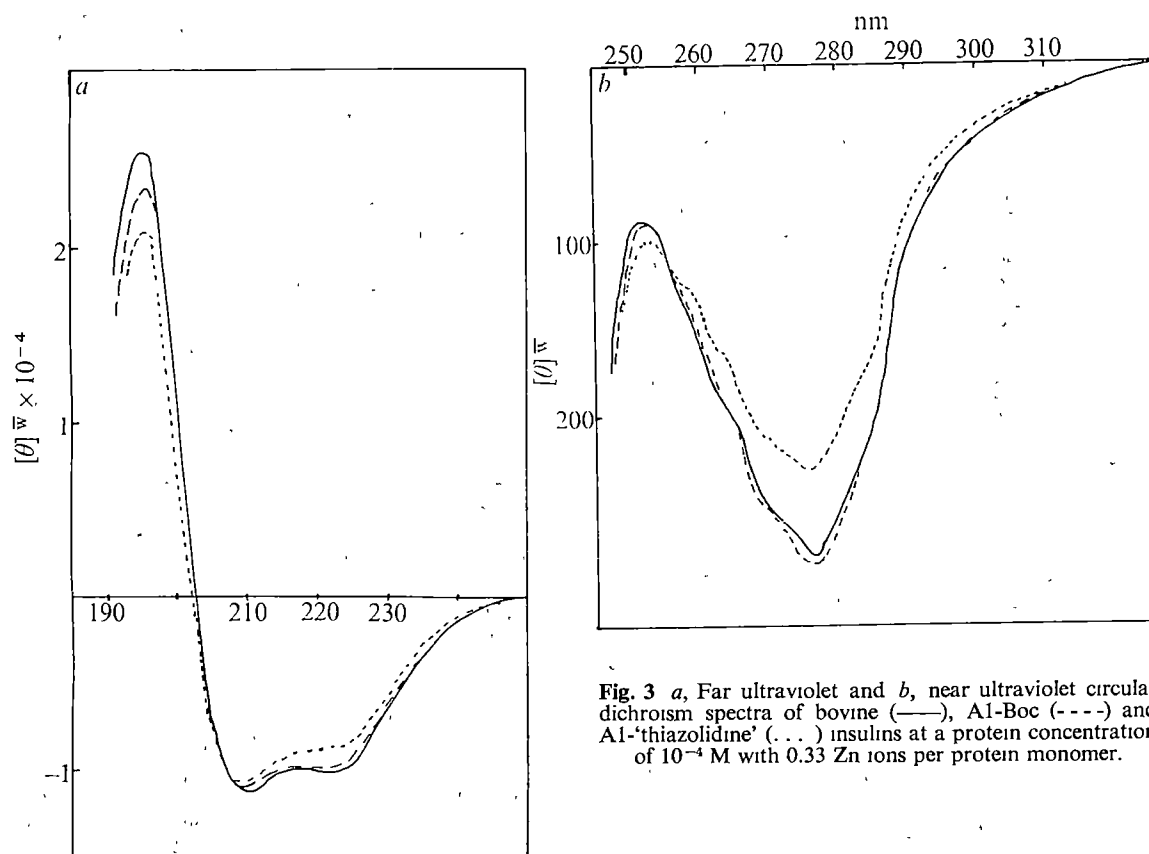
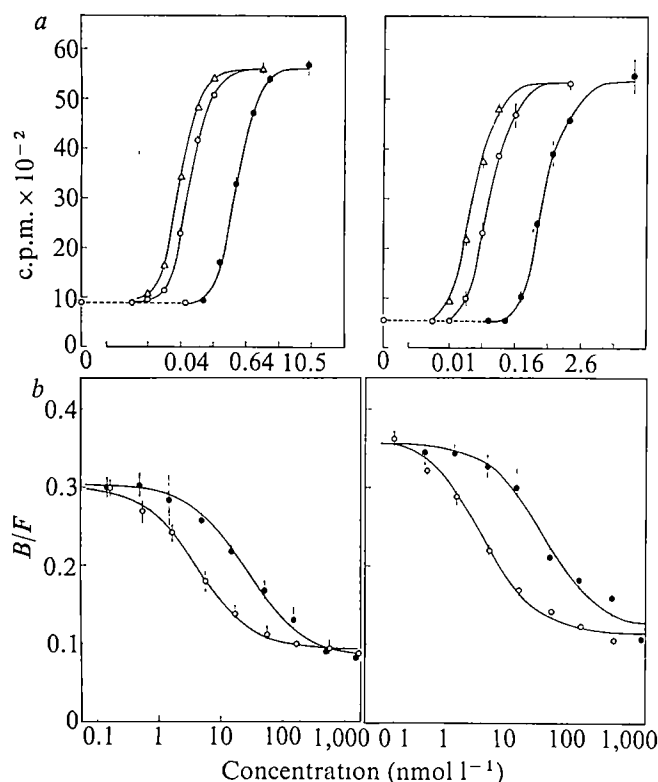


Fig. 3 a, Far ultraviolet and b, near ultraviolet circular dichroism spectra of bovine (—), A1-Boc (---) and A1-'thiazolidine' (···) insulins at a protein concentration of  $10^{-4}$  M with 0.33 Zn ions per protein monomer.





**Fig. 4** *a*, Potency of A1-*t*-Boc insulin (left) and A1-'thiazolidine' insulin (right). The ordinate denotes the conversion of 3-<sup>3</sup>H-glucose (0.5 mM, 0.2 mCi mmol<sup>-1</sup>) to lipids in fat cells after incubation for 2 h at 37 °C. The potency of the derivatives (●) relative to native insulin (○) was calculated as

$$(\text{'K}_{\text{action}} \text{ insulin} / \text{'K}_{\text{action}} \text{ derivative}) \times 100$$

The effect of addition of derivative to native insulin in the concentration ratio of 5:1 for A1-*t*-Boc insulin and 10:1 for A1-'thiazolidine' insulin is shown as Δ. The smooth curve running through these symbols is the expected addition curve for a full agonist with the potency as calculated from the experiment, that is 15.6% for A1-*t*-Boc insulin and 10.8% for A1-'thiazolidine' insulin. Each point represents the mean of four replicates ± s.d. *b*, Binding affinity of A1-*t*-Boc insulin (left) and A1-'thiazolidine' insulin (right). The ordinate denotes the ratio <sup>125</sup>I-insulin bound per l of fat cells/<sup>125</sup>I-insulin per l of medium. The <sup>125</sup>I-insulin was present in a concentration of 0.7 nM and the abscissa indicates the concentration of native insulin (○) or derivative (●). Each point represents the mean of six replicates ± s.d. The binding affinity of the derivatives relative to native insulin was calculated as

$$(K_i \text{ insulin} / K_i \text{ derivative}) \times 100$$

where  $K_i$  (the inhibition constant) was determined as described<sup>16</sup>. In the experiments shown the relative binding affinities were (with 95% confidence limits) 14.8% (13.7–16.0) for A1-Boc insulin and 10.2% (9.6–10.9) for A1-'thiazolidine' insulin.

maximal activity ( $K_{\text{action}}$ ) and the concentration which causes half-maximal displacement of specific binding of <sup>125</sup>I-insulin ( $K_d$ ) is about 1:50 both for native insulin and for the derivatives. Since the specific binding sites are believed to mediate the stimulation of lipogenesis from glucose<sup>19</sup> it follows that a derivative-receptor complex causes the same effect as an insulin-receptor complex.

## Receptor-binding region

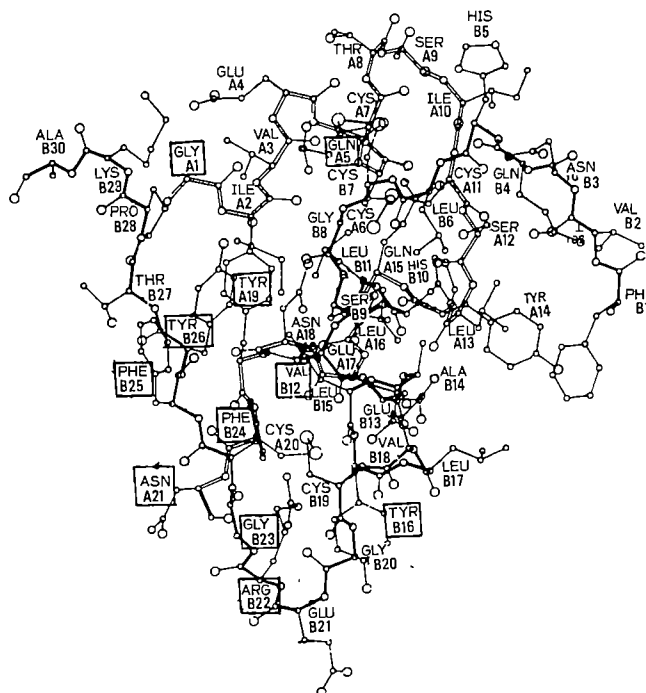
In summary, chemical modification at A1 leads to a loss of the free α-amino group which is mainly positively charged in native insulin at physiological pH. This may account to some extent for the reduced biological potency of the modified insulins, in particular A1-acetyl-insulin. X-ray analysis, however, shows that addition of larger modifying groups at A1 results in changes in the structure of the helix A1–A7 and movement of the side

chain of tyrosine A19, which in turn give rise to distortions in the arrangement of the B-chain residues involved in dimerisation. The changes are similar in A1-Boc-insulin and A1-'thiazolidine' insulin, but are more pronounced in the latter. The X-ray precession photos of A1-acetyl-insulin indicate the presence of small but similar changes in this derivative also. These differences of conformation probably contribute to the variation of dimerisation, receptor binding and potency among the modified insulins; direct steric hindrance may also contribute to decreased receptor binding, but X-ray analysis shows that it cannot account for the decreased ability to dimerise.

The results are consistent with a model in which hydrophobic residues such as B24 Phe, B25 Phe, B26 Tyr, B12 Val and B16 Tyr are involved in receptor binding in a manner similar to their involvement in dimerisation. The affinity constant for receptor binding ( $10^9$ ), however, is much greater than the dimerisation constant ( $10^5$ ). Therefore, additional interactions must exist. The proposed receptor-binding region is indicated in Fig. 5. It includes residues such as A1 Gly, A19 Tyr and A21 Asn which play no direct part in dimerisation. Their involvement is also consistent with the low receptor binding of pro-insulin<sup>3,16</sup>. If this is correct, the B25 Phe side chain—in contrast to dimerisation—would be completely buried in the insulin-receptor complex.

Residues A1, A4, A19 and A21 are probably on the periphery of the receptor-binding region. For A1 this is consistent with the results on receptor binding and potency of modified insulins<sup>5,18</sup>; the chemical nature of the modifying group can sometimes be varied with little change in the reduction of potency. A19 Tyr may be partly buried when insulin is bound to the receptor although the consensus of results on receptor binding of monoiodinated insulins indicates that the affinity is not greatly reduced<sup>20</sup>.

Certain residues outside the region proposed may also come close to the receptor. It is interesting that turkey and chicken insulins which have a histidine at A8 have considerably enhanced receptor binding<sup>21,22</sup>. A8 lies just outside the putative receptor-



**Fig. 5** The residues of the proposed receptor-binding region of insulin are indicated by a box enclosing the residue number. They include those residues on the surface of the molecule involved in dimerisation such as the residues B24–B26, and B12 and B16 in addition to the residues such as A1 and A21 which play no role in the association of insulin molecules. The diagram of the three-dimensional structure of the insulin molecule is taken from ref. 20.

binding region, and variation of this residue between threonine and alanine in porcine and bovine insulin has no effect on receptor binding. It is possible that histidine, which is larger than alanine (bovine) or threonine (porcine), forms further stabilising interactions with receptor residues and so increases the affinity of the receptor for the insulin. But it is also possible that the presence of the histidine induces a small rearrangement in the structure of the receptor-binding residues which increase the affinity. Further structural studies are required to resolve this point.

It is an attractive idea that the receptor binding should in part be analogous to dimerisation, involving a sheet hydrogen bond system between main chain functions as well as hydrophobic interactions of side chains. Hydrogen bonds in an apolar environment would contribute considerably to the stability of the hormone-receptor complex. This emphasises the critical role of the three-dimensional structure in maintaining the arrangement of these groups and would provide a mechanism for attaining a correct relative orientation of insulin and receptor.

The model for insulin receptor binding has three important consequences. First, a change in the three-dimensional structure would decrease receptor binding; this is certainly consistent with these results and with the low receptor binding<sup>23</sup> of des A21 Asn des B30 Ala insulin<sup>24</sup> and guinea pig insulin<sup>22, 23, 25</sup>, which have very disturbed structures although they retain the B-chain residues thought to bind the receptor. Secondly, full receptor binding is attained by interaction of several insulin residues with the receptor, and loss of any one residue would not be expected to abolish receptor binding; rather it would decrease it as in the case of des-pentapeptide B26-30 insulin, for example<sup>18, 26, 27</sup>. Finally, it implies that receptor binding is additive through hydrophobic interactions and formation of specific hydrogen bonds. The importance of hydrophobic interactions may be rather general in hormone receptor binding; certainly it is important in the related hormone glucagon.

This model does not preclude the possibility that certain areas within the receptor-binding region have different roles. It is possible that the interaction of certain residues of the hormone with the receptor contribute little to the stability of the insulin-receptor complex but nevertheless are relatively important to the biological response. This cannot be true of A1 as reduction of potency is paralleled by a reduction of

receptor binding. Further, De Meyts *et al.*<sup>28</sup> suggested that the residues involved in intradimer contacts are critical for the phenomenon of negative cooperativity although residues in the region of A1 are not.

As the organisation of the receptor binding region becomes better defined, the intriguing possibility, is that it may soon be possible to mimic this region in an analogue, that is more easily synthesised commercially than is the complex two-chain insulin protein.

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# Bursal dissections and gill pouch hormones

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*The terrestrial survival of birds and mammals depends on evolutionary changes in cloacal bursae and gill pouches. Comparative dissections of these pouches provide insight into the coordinated functions of cervical endocrine organs, especially as they affect lymphoid tissue.*

WHEN the lungs develop during vertebrate embryogenesis, the five paired gill pouches sequentially cease absorbing oxygen to supply epithelia for other cervical organs as follows. The first pouches supply the tympanic cavities and eustachian tubes. The second pouches supply the lateral pharynx and palatine tonsils. The third pouches supply the thymus and inferior parathyroid glands. The fourth pouches supply the superior parathyroid glands, and the fifth pouches supply the ultimobranchial bodies. In each case the

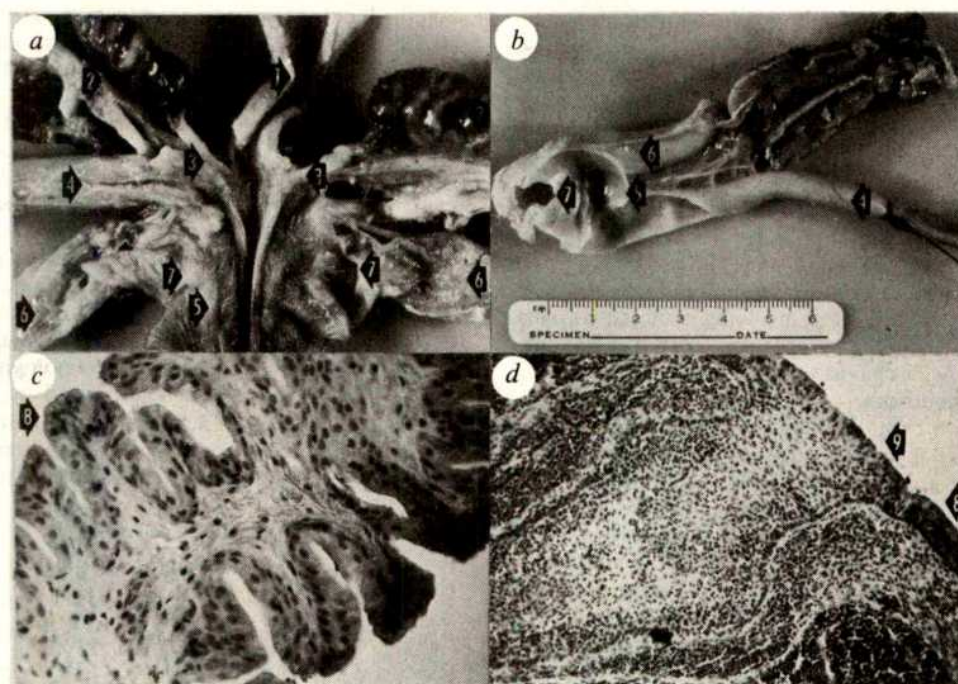
gill pouch endoderm invaginates into the cervical mesenchyme, respectively producing the hollow eustachian tubes, the palatine tonsillar crypts and the stranded epithelium of paired or fused thymus glands, paired parathyroid glands and paired ultimobranchial bodies which fuse with the thyroid primordium derived from the midline endostyle<sup>1</sup>.

## Bursal dissections

During the embryogenesis of freshwater turtles and birds gill pouch evolution occurs in this way, but in addition, cloacal pouches called cloacal bursae develop. Their structures resemble derivatives of the second to fourth gill pouches.

Dissection of the paired cloacal bursae in freshwater turtles (Fig. 1a and c) reveals pouches opening to the cloaca immediately caudal to the ureteral orifices. The





**Fig. 1** *a*, Male freshwater turtle (*Cyclemis dentata*). The cloaca (5) is opened in the dorsal midline and attached structures are dissected to expose from above downward: the bladder (1) opening into the cloaca in the midline, the paired kidneys (2) and ureters (3) which open into the cloaca, the colon (4) incised but not opened to the left and the paired cloacal bursae (6) opening into the cloaca caudal to the ureters and the colon at (7). *b*, Pekin duckling with the kidneys to the right and the right ureter coursing over the cloacal bursa of Fabricius (6). The right side of the lower colon (4) and cloaca (5) are resected to expose the opening of the bursa into the cloaca just above the anus and caudal to the ureter (7). *c*, In the turtle bursa the pseudostratified columnar epithelium (8) evaginates into the lumen like gill folds in fish. Submucosal lymphoid tissue is inconspicuous. *d*, In the avian bursa the pseudostratified columnar epithelium (8) invaginates (9) into the submucosa to produce epithelial sheets which are infiltrated with lymphocytes and surrounded by dense lymphoid tissue.

pouches are lined by pseudostratified columnar epithelium similar to that in the gill pouch orifices of fish and tadpoles. The epithelium folds into the underlying connective tissues. Subepithelial lymphoid tissue is sparse.

Dissection of the single cloacal bursa (bursa of Fabricius) in birds (Fig. 1*b* and *d*) reveals a midline pouch open to the cloaca immediately caudal to the ureteral orifices. The pouch is lined by pseudostratified columnar epithelium which folds into the lumen, and invaginates into underlying connective tissue to form bottle-necked epithelial sheets infiltrated with lymphoid cells, surrounded by dense organised lymphoid tissue. This 'lympho-epithelial tissue' resembles the thymus gland of bony fish, tadpoles, turtles, birds and mammals; but differs in that its epithelium does not become separated from that lining the gut lumen and it does not contain Hassall's corpuscles, epithelial cysts, myoid cells or clusters of parathyroid cells (similar to those common in turtle thymus). The avian bursa also resembles the palatine tonsils in that the epithelium invaginates deeply, does not become separate from gut luminal epithelium, is heavily infiltrated with lymphoid cells and is surrounded by dense organised lymphoid tissue. It differs from the palatine tonsils (but not the adenoids) in that the surface epithelium is columnar instead of squamous; and in that germinal centres do not develop in the surrounding lymphoid tissue.

These dissections suggest that the cloacal bursae in freshwater turtles and the avian bursa of Fabricius are homologous structures which, in turn, share homologies with the second to fourth gill pouches.

## Bursal functions

In 1590 Hieronymus Fabricius<sup>2</sup> described the cloacal bursae in birds. In 1733 Perrault<sup>3</sup> recorded their presence in some species of turtles. In 1914 Jolly<sup>4</sup> suggested that such "anal sacs" in turtles are phylogenetic precursors of avian

bursae. Functions of these bursae remained obscure until the 1950s.

In 1958 Smith and James<sup>5</sup> reported that paired cloacal bursae are rudimentary or lacking in terrapins and sea turtles, but well developed in freshwater turtles which normally hibernate underwater at temperatures less than 40–60 °F. They concluded that freshwater turtles use these bursae as gills when water is flushed in and out of the cloaca during hibernation. Having low basal metabolic rates, especially during hibernation, freshwater turtles (especially Emydidae) derive sufficient dissolved oxygen through cloacal respiration to survive through the winter under ice-covered water<sup>5–7</sup>. In Pleurodira the cloacal bursae are partially fused<sup>8</sup> (resembling the single avian bursa). Soft-shelled turtles (for example, *Trionyx triunguis*) lack cloacal bursae, but facultatively respire underwater through the epidermis or through pharyngeal gills<sup>7</sup> (in the second gill pouch position where mammalian tonsils develop). Retaining functional gills either in the pharynx or in cloacal bursae, the freshwater turtles (like tadpoles) are pivotal in the evolution of respiration and associated adaptations essential to survival on land.

In 1956 Glick *et al.*<sup>8</sup> reported that surgical bursectomy in newly hatched chicks results in deficient antibody production. Their observation established that the single bursa of Fabricius has an important immunological role in neonatal birds. Moreover, it revolutionised modern concepts of mammalian immunology when the effects of testosterone bursectomy were compared with those of surgical thymectomy in newborn rodents<sup>9–11</sup>. The new concepts<sup>11–13</sup>, especially those concerning bursa-dependent B cells, are open to criticism for diverse reasons<sup>14</sup>, some of which are as follows. (1) Mammals lack a caudal anatomical counterpart of the avian cloacal bursa<sup>13,14</sup>. (2) In human babies with "third and fourth pouch syndromes" wherein the thymus and the parathyroid glands failed to develop, in addition to tetany

and various cervico-facial anomalies, variable defects in immunoglobulin production are evident<sup>15,16</sup>. (3) Surgical bursectomy in chicks on the fourth day of incubation (before the cloaca opens into the chorio-allantoic sac) does not completely suppress immunoglobulin production in surviving chicks, and usually suppresses thymic development<sup>17</sup>. Testosterone bursectomy in hatching chicks also suppresses thymic development<sup>17</sup>, while testosterone produces thymic involution in mammals<sup>18-20</sup>. Bursectomy in birds and thymectomy in mammals do not cause serious immunological defects a few weeks after birth<sup>8,10</sup> once a critical extrathymic lymphoid tissue mass<sup>21</sup> is established with thymic involution<sup>14,20</sup> and normal foraging<sup>14</sup>.

(4) Among other differences, birds differ from mammals in that they lack tonsils with germinal centres and intestinal Peyer's patches<sup>4,22</sup>. Birds also lack maternal immunoglobulin passed through the placenta before birth and from milk after hatching. In mammals the tonsillar and Peyer's patch germinal centres commence development with the onset of independent breathing and eating<sup>14,22</sup>. After development of such germinal centres, endogenous antibody production commences in quantity<sup>13,23</sup>. Whereas the tonsillar crypts develop such that they may trap newly ingested food along with airborne or food-borne bacteria, the avian bursa is oriented to 'trap colonic contents' containing the undigested or 'commensal' remains. In addition, the avian bursal epithelium demonstrates unique capacities for the pinocytotic digestion of colloidal intestinal contents, while the bursal musculature contracts in synchrony with respiration<sup>24</sup>. Such distinctive anatomico-physiological arrangements may be cogent to neonatal antibody production in the respective species.

(5) Implantation in Millipore diffusion chambers has shown that thymic and bursal effects on lymphoid tissue development and immunity in mammals and birds are

mediated by hormones produced in associated epithelial cells<sup>21,26-28</sup>. Although distinctive bursal hormones remain to be isolated, a thymic-epithelial hormone (thymosin) with reproducible lymphocytopoietic activity has been partially purified<sup>29,30</sup>, and corrects immunological and nutritive deficiencies in neonatally thymectomised animals and congenitally athymic human babies<sup>31</sup>. Irrespective of species, many, if not all thymic relationships to body growth, immunity and stress can be explained simply in terms of thymosin's trophic action on DNA metabolism in local and distant lymphoid tissue<sup>14</sup>.

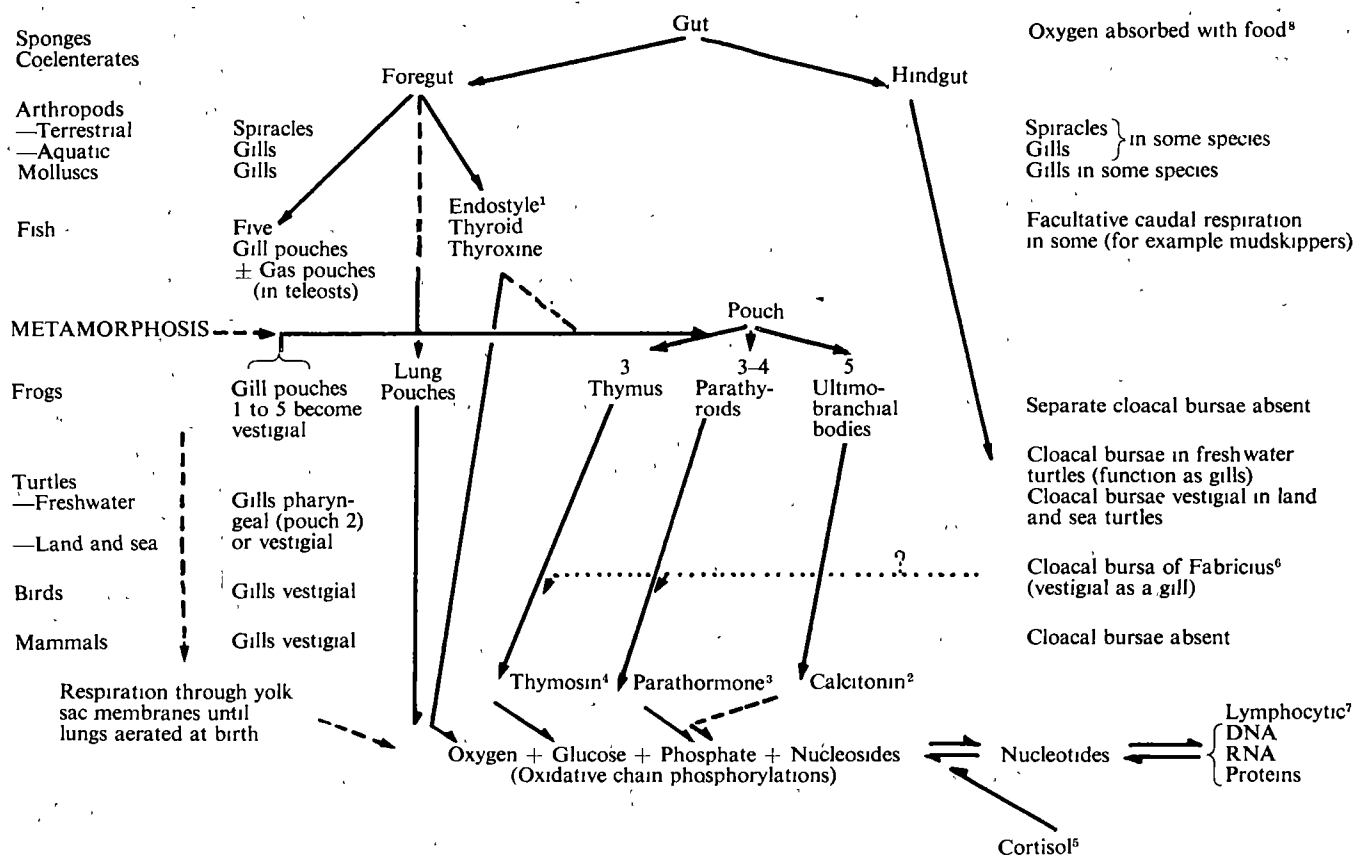
(6) Studies<sup>32,33</sup> of poikilothermic vertebrates (fish, amphibia, reptiles) reveal evidence of humoral immunity and immunoglobulin production (chiefly IgM) from lymphocytes in thymus, spleen and kidneys before bone marrow develops, and in the absence of cloacal bursae in all species except temperate (as opposed to tropical<sup>15-7</sup>) freshwater turtles. As Jordan<sup>22,34</sup>, Marine<sup>19</sup> and Cooper<sup>32</sup> pointed out, attention to gill and thymic evolution in anuran amphibians (especially larval frogs) may supply promising avenues of research into the endocrine system.

Thus it can be surmised that in several species of freshwater turtles the cloacal bursae function like gill pouches in fish and tadpoles. In birds which are close phylogenetic relatives of turtles, the cloacal bursa appears to function like an organ hybrid from the second to fourth gill pouch derivatives in mammals, that is the tonsils, thymus and parathyroid glands; and an organ which may contract like gills in synchrony with respiration.

## Respiration and cervical endocrine evolution

In embryonic birds and mammals when the third to fifth branchial pouches undergo metamorphosis into cervical endocrine glands and the lung pouches are developing, res-

Fig. 2 Evolution of entodermal pouches concerned with respiration.





piration continues through the vitelline membranes within the liquid environment of the egg or the uterus<sup>1</sup>. When breathing under water suddenly changes to lung breathing with hatching or birth, the cervical endocrine epithelia continue to produce hormones which accelerate the internal metabolism of oxygen, glucose and phosphate, especially within lymphoid tissues<sup>14</sup>. The teleological relationships are tentatively outlined in Fig. 2.

The superscript numbers in Fig 2 are interpreted as follows. (1) The thyroid gland, derived from the endostyle<sup>1</sup>, produces thyroxine which accelerates metamorphosis<sup>22,34</sup> and the rate oxygen is consumed to create high energy phosphate bonds in nucleotides<sup>35</sup>, especially in lymphoid tissues<sup>14,35</sup>. Thus the rates of DNA synthesis and growth in lymphoid tissues parallel the basal metabolic rate during phylogenesis<sup>14,36</sup>, ontogenesis<sup>22,34</sup>, puberty<sup>36</sup>, alimentation<sup>37</sup>, and hyperthyroidism<sup>14,35</sup>, irrespective of the growth rate in other tissues.

(2) The ultimobranchial bodies evolve into the parafollicular cells of the thyroid gland which produce calcitonin<sup>35</sup>.

(3) Parathormone and calcitonin govern phosphate availability and calcium ion concentrations potentially favourable to oxidative chain phosphorylations<sup>35</sup>. Parathormone stimulates growth of lymphoid tissue, especially in thymus<sup>18,19</sup>. Also it affects the oxygen affinity of erythrocytes by actions on the phosphates of 2,3-diglycerolphosphate and adenosine diphosphate<sup>38</sup>.

(4) Thymosin accelerates nucleic acid synthesis, especially in lymphocytes<sup>29,30</sup> which grow faster, contain higher concentrations of DNA, and release DNA more readily than other cells in the body<sup>14</sup>. Because of its insulin-like effect on lymphoid tissue<sup>38</sup> and because its action diametrically opposes that of cortisol<sup>14</sup>, thymosin's action appears to be on the glucose moiety in oxidative chain phosphorylations. Parallel to thyroxine, it seems to increase the high energy nucleotide-bound phosphate yield per quantity of glucose consumed, particularly in lymphoid tissues. (5) Cortisol interferes with the utilisation of glucose in oxidative chain phosphorylations essential to lymphocytic DNA and protein synthesis<sup>39-43</sup>. Characteristically it engenders lysis rather than synthesis of DNA in lymphoid tissues, especially in the thymus gland<sup>14,43-46</sup>.

(6) If the "third and fourth pouch syndromes"<sup>15,16</sup> in immunodeficient human babies are clues, hormones from the avian cloacal bursa seem to have actions like those of thymosin plus variable quantities of parathormone. The combined actions seem to foster RNA synthesis essential to immunoglobulin production, as well as the prerequisite DNA synthesis and formation of appropriate messenger RNA.

(7) Together with lymphotropic actions, thyroxine, thymosin, parathormone and calcitonin exert others essential to survival in vertebrates destined to crawl out of the water, breathe air and move on land. Actions on bone calcification and erythrocytopoiesis are especially important not only after birth, but also during metamorphosis when sequentially: the thymus, parathyroid and ultimobranchial bodies develop out of the gill pouches<sup>1</sup>; the lung pouches commence development<sup>1</sup>; the cartilaginous skeleton commences calcification to stiffen the spine and provide 'a relatively rigid thorax', calcifying cartilages are invaded by erythrocytopoietic mesenchyme<sup>1,22</sup>, while the limbs develop to bear weight; haemoglobin structure changes to carry more oxygen<sup>12,22,34</sup> organised lymphoid tissues commence to grow in a periarteriolar environment of relatively high oxygen tension<sup>14,47,48</sup>.

With the establishment of intramedullary erythrocytopoiesis and the attendant enucleation of erythrocytes in mammals<sup>12,22</sup>, lymphocytes multiply around the arterioles in the thymus, spleen, nodes, marrow and intestine to become the most common nucleated cells and constitute 1-3% of the total body mass in the healthy, well fed mammal<sup>14,36</sup>. Hypoxia engenders sudden lymphocytolysis with shrinkage

of the lymphoid tissue mass in minutes or hours<sup>14,48-49</sup>. Starvation (eliminating the exogenous supply of glucose, phosphate and precursors for nucleosides) causes lymphocytolysis with shrinkage at a slower rate, but at a rate far exceeding that of cellular attrition in the liver or other tissues<sup>14,45-50</sup>. The lymphocytolytic effects of anoxia and starvation are mediated by adrenal glucocorticoids<sup>14,45,46</sup> which make lymphocytic proteins available for gluconeogenesis<sup>43,46</sup> and release nuclear material rich in high energy nucleotides, especially during stress<sup>14</sup>.

(8) The invertebrates and fish are included in the diagram to indicate various forms of respiration which take place during vertebrate phylogenesis<sup>51</sup>, as well as mammalian embryogenesis.

If accurate, these teleological correlations based on comparative pouch dissections will necessitate revisions in immunological theory and provide new insights into endocrinology. In turn, when thymosin, parathormone and calcitonin become available in quantity, as are thyroxine and adrenal glucocorticoids, increasingly rational hormonal treatment of diseases involving bones, erythrocytopoietic and lymphocytopoietic tissues may follow.

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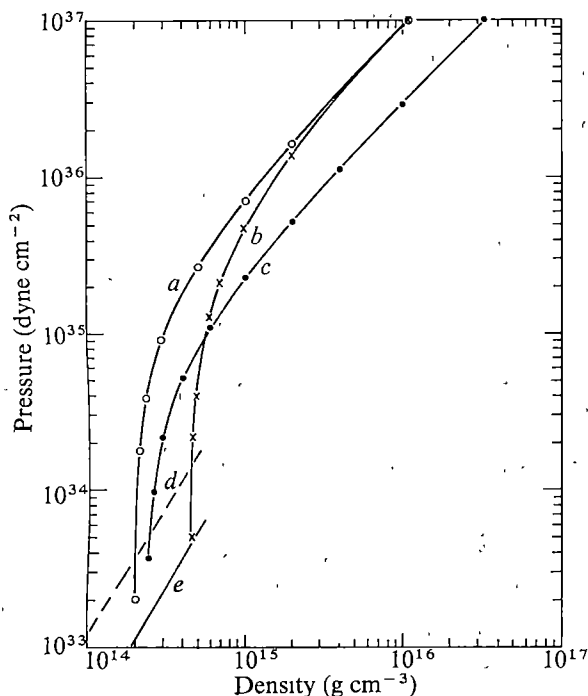
# letters to nature

## Obese 'neutron' stars

WE show here that non-rotating, spherically symmetric, general relativistic 'neutron' stars of mass  $\geq 3M_{\odot}$  are consistent with the known laws of physics. In particular, for the 'bag' model of hadrons, we find  $M_{\max} \approx 3M_{\odot}$ . The maximum mass for polytropic equations of state is found to be  $\approx 5M_{\odot}$ .

This is particularly interesting since it has been repeatedly claimed<sup>1</sup> that the X-ray source Cygnus X-1 is a binary star system containing a black hole accreting matter from its relatively normal companion star HDE226868. The arguments leading to this conclusion, while requiring many assumptions (for example that Cyg X-1 is a binary rather than a triple star system; that the X-ray source is powered by accretion and is not, say, the result of the radiation of spin energy of a rapidly rotating white dwarf; that general relativity is correct beyond its tested domain) depend most crucially on the existence and value of an upper mass limit for 'neutron' stars. (Throughout this discussion the term 'neutron' star is used to refer to a stable matter configuration of mean density  $\approx 10^{14}$ – $10^{15}$  g cm<sup>-3</sup>, whether the system is predominantly composed of neutrons, quarks, or some other hypothetical state of matter.) Estimates<sup>2,3</sup> for the mass of Cyg X-1 range from  $2.6M_{\odot}$  to as much as  $15M_{\odot}$ . Most recently, Bahcall<sup>4</sup> has suggested a value of  $M \approx 4.7 (d/2)^2 M_{\odot}$ , where  $d$  is the distance to the object in kpc. Since it seems that  $d \approx 2 \pm 0.5$  kpc, one has  $M_{\text{Cyg}} \approx 3$ – $7M_{\odot}$ . Based on the claim<sup>5</sup> that the absolute upper mass limit for non-rotating, general relativistic neutron stars is  $3.2M_{\odot}$  whatever the equation of state of matter at supernuclear densities, many authors have concluded that Cyg X-1 must be a black hole. Because of the significance of this result, we have examined

**Fig. 1** Equations of state: *a*, extreme causal equation;  $p = (\rho - 2 \times 10^{14})c^2 + P_m$ ; *b*, equation producing Rhoades-Ruffini result;  $p = (\rho - 4.6 \times 10^{14})c^2$ ; *c*, quark bag model;  $p = (\frac{1}{3})(\rho - 2.28 \times 10^{14})c^2$ ; *d*, free Fermi gas of neutrons; *e*, an extension of Negele's nuclear model.



the dependence of the neutron star upper mass limit on assumed properties of high density matter.

We consider here only the solutions to the hydrostatic equilibrium problem for spherically symmetric, non-rotating, general relativistic neutron stars. The proper gravitational mass  $M(r)$  inside a radial distance  $r$  is given by

$$M(r) = \int_0^r 4\pi r'^2 \rho(r') dr' \quad (1)$$

where  $\rho(r)c^2$  is the total (matter and field) local energy density. The equation of hydrostatic equilibrium relating the pressure  $p(r)$  to  $\rho(r)$  and  $M(r)$ , the Oppenheimer-Volkoff equation<sup>6</sup>, is

$$\frac{dp}{dr} = -\frac{GM(r)\rho(r)}{r^2} \left[ 1 + \frac{p(r)}{\rho(r)c^2} \right] \left[ 1 + \frac{4\pi r^3 p(r)}{M(r)c^2} \right] \left[ 1 - \frac{2GM(r)}{rc^2} \right]^{-1} \quad (2)$$

Given an equation of state

$$p = p(\rho) \quad (3)$$

and specific boundary conditions (central density and surface pressure or density) a unique model arises.

For most equations of state  $p = p(\rho)$ , the hydrostatic equilibrium equation cannot be integrated analytically and so must be evaluated numerically. One simple analytical case, however, demonstrates two of the results we have found numerically. If  $p = \alpha \rho c^2$ , then a closed form solution for the case of infinite central density is

$$\rho(r) = (c^2/2\pi G)[\alpha/(\alpha^2 + 6\alpha + 1)](1/r^2). \quad (4)$$

Taking this equation to be valid above some density  $\rho_s > \rho_N$  (where  $\rho_N = 2 \times 10^{14}$  g cm<sup>-3</sup> is a characteristic nuclear density) one has for the 'core' of a model neutron star (matched to an outer envelope with a 'realistic' nuclear equation of state, but which in general contains no more than  $0.1M_{\odot}$ ) a mass

$$M = (c^3/G^{3/2})(2/\pi)^{1/2}[\alpha/(\alpha^2 + 6\alpha + 1)]^{3/2}(1/\rho_s^{1/2}). \quad (5)$$

We immediately see that for  $p = \alpha \rho c^2$ ,  $M$  is maximised when  $\alpha = 1$ , that is, for  $dp/d\rho = c^2$ . We note further that  $M$  depends on the inverse square root of that density above which the equation of state  $p = \alpha \rho c^2$  applies. We find that for  $\alpha = 1$  and  $\rho_s = \rho_N$ , one has  $M_{\text{core}} \approx 2M_{\odot}$ .

The real equation of state of matter at densities  $\geq 2 \times 10^{14}$  g cm<sup>-3</sup> is unknown. Something can, however, be deduced from known properties of several neutron stars. The mass<sup>7</sup> of the X-ray emitting object Her X-1, probably a neutron star, seems to be  $1.3 \pm 0.1M_{\odot}$ . The binary star system<sup>8</sup> containing the pulsar PSR1913+16 has a total mass  $2.9M_{\odot}$ . If, as seems plausible on evolutionary grounds<sup>9</sup>, the unseen companion is a white dwarf (which must be slowly rotating with a mass  $\lesssim 1.4M_{\odot}$ ), then the pulsar (neutron star) mass is  $\geq 1.5M_{\odot}$ . Most recently<sup>10</sup> the binary X-ray source Vela X-1, with a pulse period of  $\approx 283$  s, has been found to have a most probable mass of  $\approx 1.7M_{\odot}$ . Numerically integrating equations (1) and

(2) with the central density as a parameter and assuming that neutrons form a cold, perfect Fermi gas up to arbitrary density, Oppenheimer and Volkoff<sup>6</sup>, and later Misner and Zepolsky<sup>11</sup> found the maximum mass to be  $\approx 0.7M_{\odot}$ . We can now conclude that matter at supernuclear densities, at least in some neutron stars, does not behave as a free Fermi gas of neutrons. Neither does a soft-core potential<sup>12</sup> describe neutrons at high densities, since it yields a value of  $M_{\max}$  as low as  $0.27M_{\odot}$ . Other equations of state, such as that of Malone, Johnson and Bethe<sup>13</sup> (with  $M_{\max} \approx 1.85M_{\odot}$ ) have upper mass limits only just consistent with the observed masses of neutron stars.

In view of the uncertainties in  $p(\rho)$ , we have numerically investigated some hypothetical equations of state in order to get a better feel for what  $M_{\max}$  depends on. We chose an equation of state to be either a free Fermi gas of neutrons or a more realistic equation<sup>14</sup> of state which accounts for the attractive part of the nuclear potential up to a density  $\rho_s$  (the final result is quite insensitive to this choice). For  $\rho \geq \rho_s$ , polytropic equations of state were chosen of the form

$$p = \beta \rho^{\gamma} + p_0 \quad (6)$$

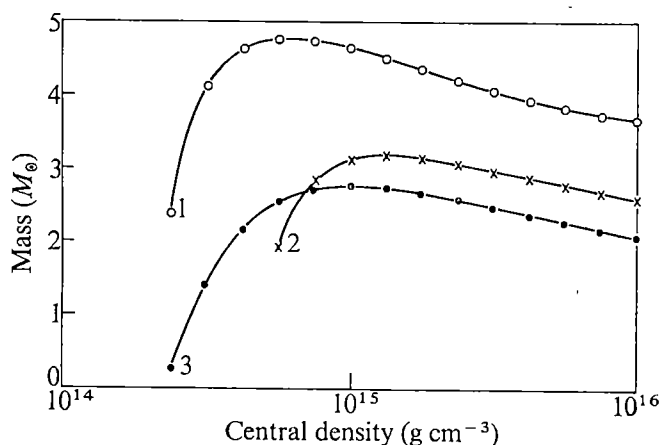
with  $1 \leq \gamma \leq 5/2$  and with a range of values of  $\beta$  which includes values for which  $dp/d\rho > c^2$ . The hydrostatic equilibrium equation was then numerically integrated for given values of  $\beta$ ,  $\gamma$  and  $p_0$ , with the central density as a parameter.

The main result of these calculations was that for given values of  $\beta$  and  $\gamma$  (with  $p_0 = 0$ ),  $M_{\max}$  varied approximately as  $1/\rho_s^{1/2}$ , as in the analytical case with  $p_{\text{cent}} = \infty$ . The greatest value of  $M_{\max}$  for  $dp/d\rho_{\text{cent}} \leq c^2$  was reached for the case which smoothly matched  $p = \rho c^2$  to the 'true' nuclear equation of state at  $\rho_s = \rho_N = 2 \times 10^{14} \text{ g cm}^{-3}$ , keeping both the pressure and density continuous across the change of state (no 'phase' transition), that is

$$p = (\rho - \rho_N)c^2 + p_M \quad (7)$$

(see Fig. 1, curve *a*). The resulting plot of the mass against the central density is shown in Fig. 2 (curve 1). It can be seen that the curve has a maximum at  $\sim 4.8M_{\odot}$ . (This agrees with the results for the same model found independently by others<sup>13,15</sup>.) Such a star is stable and has an equation of state consistent with everything known about nuclear physics at or below nuclear density. The difference between this mass limit and the one found by Rhoades and Ruffini<sup>5</sup> (curve 2 in Fig. 2) arises almost entirely from the choice of matching density and is just the factor  $(\rho_r/\rho_N)^{1/2} \approx 1.5$ , where  $\rho_r = 4.6 \times 10^{14} \text{ g cm}^{-3}$  is the density to which they extrapolated the equation of state of nuclear matter above nuclear density.

Fig. 2 Mass as a function of central density for: (1) *a* matched to *d*; (2) *b* matched to *d*; and (3) *c* matched to half the free Fermion equation. Stars to the left of the peaks are stable. Matching equations of state *a*, *b* or *c* to either *d* or *e* made little ( $< 0.1 M_{\odot}$ ) difference in the resulting mass limits.



It should also be noted that, for the range of  $\gamma$  we tested the computed values of  $M_{\max}$  depended only weakly on  $\gamma$ . But, for such a polytropic equation of state, as  $\gamma \rightarrow \infty$ ,  $dp/d\rho \rightarrow 0$ , so that the fluid becomes incompressible. In this limit, for a star of uniform density  $\rho = \rho_N$ , one finds<sup>15</sup>  $M_{\max} \approx 8M_{\odot}$ .

These results gain physical significance when one considers the possibility of achieving a very stiff equation of state at or near nuclear density. In particular, the recently proposed 'bag' model of hadrons produces a stiff equation of state at low densities. In essence, for densities just above nuclear, overlapping bags of quarks act as a sea of relativistic massless (or low mass) free fermions. From the results of Degrand *et al.*<sup>16</sup> which fit bag model parameters to elementary particle properties, one finds an equation of state

$$p = \frac{1}{3}(\rho - \rho_B)c^2 \quad (8)$$

where  $\rho_B \approx 2.28 \times 10^{14} \text{ g cm}^{-3}$  is one of their two model values of  $\rho_B$ . Using this equation of state (curve *c*, Fig. 1), one finds a maximum mass of  $M_{\text{bag,max}} \approx 2.8M_{\odot}$  (curve 3, Fig. 2). Most of the mass arises from near the region  $\rho \approx \rho_B$ : the equation of state for  $\rho \gg 10^{15} \text{ g cm}^{-3}$  and for  $\rho \ll 10^{14} \text{ g cm}^{-3}$  is virtually irrelevant to the determination of this value. Such a star is, of course, no longer a neutron star but a quark star. (The possibility that supernuclear matter in 'neutron' stars consists of free quarks has also been considered by Collins and Perry<sup>17</sup>, though they did not explicitly calculate any stellar models.)

It therefore seems possible to have a stable (not fully collapsed) neutron star with a mass in the range  $3-5M_{\odot}$ . These numerical results are consistent with the results of others<sup>18</sup> (for essentially uniform density models) that massive ( $\approx 5M_{\odot}$ ), spherically symmetric, general relativistic neutron stars are in principle possible. Though the overlap between the masses of models considered here and the mass range deduced for Cyg X-1 is small, it seems too early to rule out the possibility that Cyg X-1 is not a black hole but simply a bag of quarks, or that it exists in some other unusual state of supernuclear matter.

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## Recognising simulated earthquakes

KOLAR AND PRUVOST<sup>1</sup> have suggested that explosives could be detonated to provide all the characteristics of earthquakes when they are recorded by distant seismographs. Should this experiment be confirmed the effect on efforts to solve the seismological problems which at the moment preclude satisfactory agreement on an underground nuclear test ban would be profound, we present here the results of a relevant experiment.

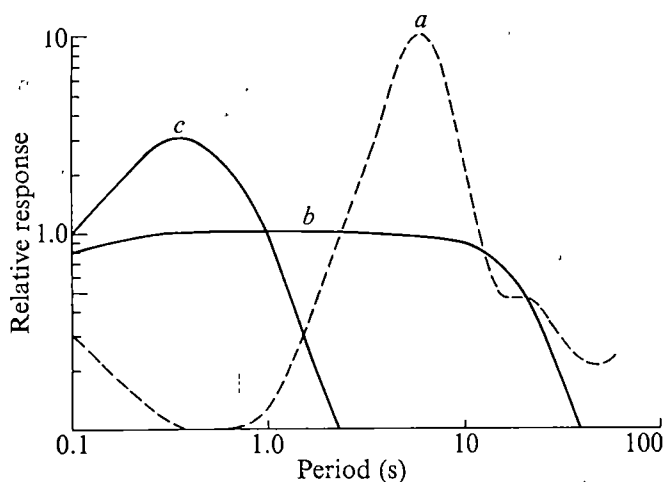
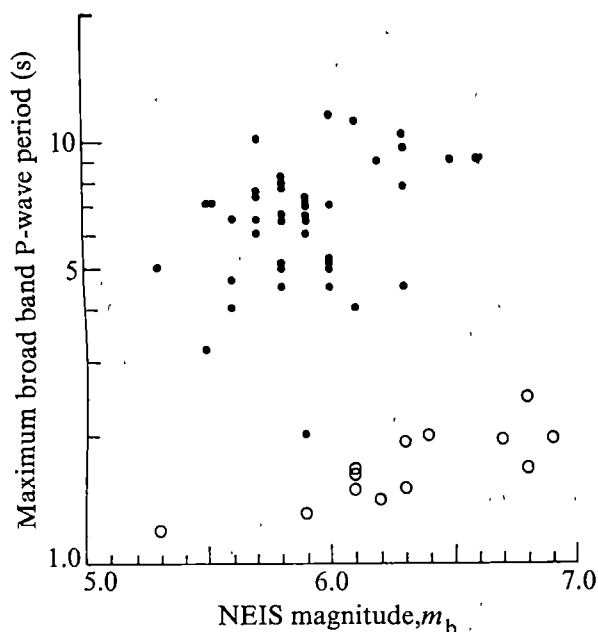


Fig. 1 Typical microseismic noise spectrum (a), (maximum, 10  $\mu$ m) superimposed on the broad-band (b) and the LRSM short period (c) seismograph response. The LRSM response peaks here at an amplification of 30,000 but may be operated at amplifications of up to 300,000. The broad band rarely operates at an amplification of more than 10,000.

What Kolar and Pruvost demonstrated was that the direction of first motion could be effectively disguised. They showed that a complex P waveform could be created, and that the conventional magnitudes,  $m_b$  and  $M_s$ , could be so manipulated that unsuspecting observers would find that all the criteria conformed with those used to establish the occurrence of an average earthquake.

For their demonstration, however, Kolar and Pruvost<sup>1</sup> used recordings made on the narrow band seismograph systems developed especially for the Long Range Seismic Measurement (LRSM) project, primarily used to detect low yield explosions. Bearing in mind Pasechnik's demonstration<sup>2</sup> of spectral differences between earthquakes and explosions we repeated Kolar and Pruvost's experiment, using a recording from a less sensitive, broader band seismograph (see ref. 3). Our diagram of the relative amplitude responses of the LRSM and broad band seismographs (Fig. 1) include a roughly drawn noise spectrum which illustrates the principal recording problem faced by seismologists. Though

Fig. 2 The maximum broad-band P-wave signal period against the short period magnitude reported by NEIS. ●, Earthquakes; ○, explosions.



the broad-band system includes the peak in the noise spectrum, it falls off at 12 dB an octave beyond a period of 12 s; it is a broad-band instrument only for body waves.

In the first half of our experiment we tested Pasechnik's assertion<sup>2</sup> that the broad-band body-wave data provide means for distinguishing between earthquakes and explosions. Though the sample is small, the data of Fig. 2 give almost total separation between the P-wave periods of two disturbances above and below the 3-s ordinate. The single exception is known from evidence of a reliable surface reflection pP wave to be an earthquake at a depth of 40 km. The second stage of the experiment consisted of repeating Kolar and Pruvost's procedure for body waves in every detail, except that a broad-band recording of an explosion was used in place of the LRSM record (Fig. 3).

No change of period between the original and mixed signal is observed, so the mixed 'event' remains in the explosion population of Fig. 2. (The experiment was also repeated with Rayleigh waves and, as expected because of the much longer period, the original and mixed signals were virtually identical in shape.) Only the P waves of the larger

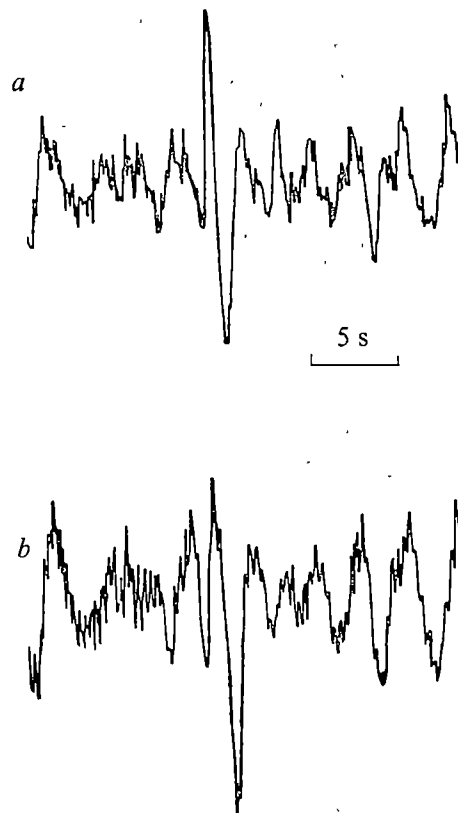


Fig. 3 a, P-wave generated by a Kazakh explosion recorded on the broad-band system and used to simulate the Kolar-Pruvost experiment; b, the resultant seismogram after summation. Note that the predominant period remains almost the same.

explosions would have been observed in this particular case; the earlier ones of the series are below noise level. Therefore, to illustrate the experiment in more detail, to recapitulate the technique, and to carry the test a little further, a synthetic, broad-band explosion signal was summed with no noise (Fig. 4) and the amplitude spectra of the original and final signal were taken (Fig. 5). The peak amplitude remains stable at 2 s; Kolar and Pruvost<sup>1</sup> designed their experiment to reduce the power at 1 s, thereby reducing the apparent body-wave magnitude for their narrow band recording.

The explosion signal shown in Fig. 3a represents a yield of 100 kt on a relatively quiet day in the UK. It implies



that the smallest explosion which could be detected by broad-band seismographs in the UK would be about 50 kt, which is about as far as earthquake simulation technique can be taken, depending as it does on several narrow-band instruments detecting the smaller (by a factor of 10) explosions.

We propose that the broad-band seismograph looks promising as a safeguard against attempts to camouflage moderately large underground tests with a carefully timed series of explosions. It seems likely that the detection level at most stations could be better than 50 kt after array processing (experiments are in hand), though according to Kondorskaya and Aranovich<sup>4</sup> the resolution of the period discriminant is likely to be weaker at lower magnitudes. Nonetheless a serious would-be violator, in allowing for the possible occurrence of exceptional recording conditions, unknown improvements in techniques and the possible existence of undisclosed stations, would have to credit

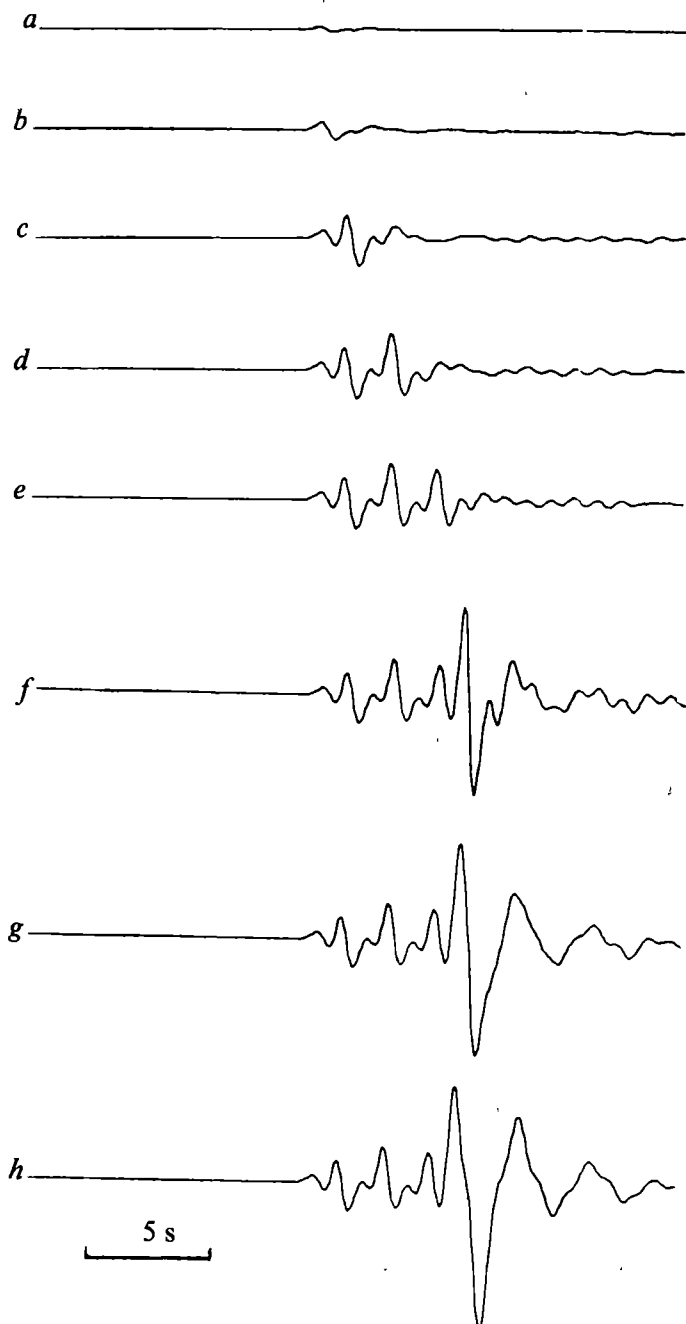


Fig. 4 Model simulation of the multiple explosion, illustrating the development of the final signal. *a*, 3 kt; *b*, + 9 kt, delayed 0.3 s; *c*, + 29 kt, delayed 1.1 s; *d*, + 29 kt, delayed 2.9 s; *e*, + 29 kt, delayed 2.7 s; *f*, + 100 kt, delayed 5.7 s; *g*, + 100 kt, delayed 6.0 s; *h*, + 100 kt, delayed 6.3 s.

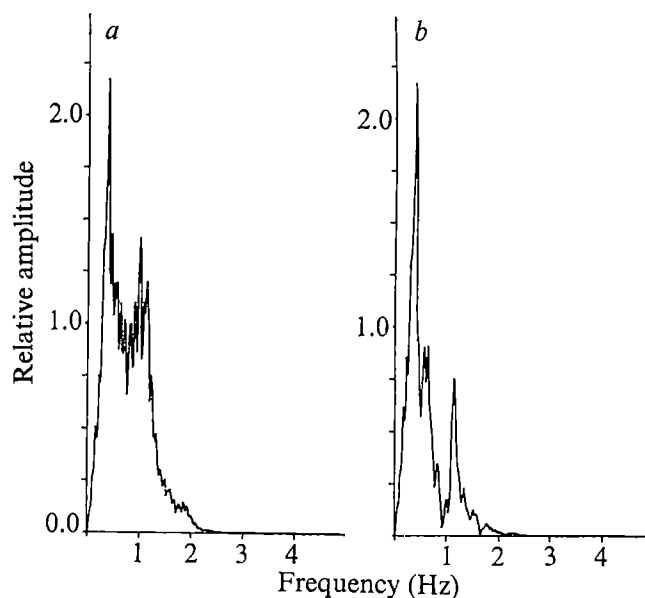


Fig. 5 *a*, Amplitude spectrum of the single explosion used in the simulation experiment; *b*, amplitude spectrum of the resultant multiple explosion. Note that the large, low frequency, spectral peak remains at the same frequency.

'national networks' with somewhat better detection capacities than publicly revealed. A safety factor of two would not be an unreasonable margin to allow. Thus, the geophysical uncertainties operate against the violator.

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## Anomalous geomagnetic field during the late Ordovician

MEASUREMENTS of the Lower Palaeozoic magnetic field in the British Isles<sup>1</sup> show that it changed from around  $D=355^\circ$ ,  $I=-50^\circ$  to  $D=20^\circ$ ,  $I=-54^\circ$  (corresponding to an apparent shift of the south palaeomagnetic pole from  $0^\circ$ W to  $35^\circ$ W along the present Equator) between Ordovician and Devonian times. Following unpublished work by W. E. Tremlett and J. C. Briden, we report here conflicting evidence from the intrusive rocks between Conway and Pwllheli, North Wales. These rocks therefore seem to show either previously unrecognised tectonic rotations or hitherto unsuspected variation in the palaeomagnetic field.

Throughout North Wales, an area of complex Palaeozoic tectonism, a large number of acid igneous intrusions occur whose ages within the Lower Palaeozoic are ill defined. Some bear strong petrogenetic affinities with the Ordovician acid extrusive rocks of the area and on field evidence there is little doubt that such intrusions as the Tan-y-Grisiau microgranite, the Myndd Mawr microgranite, the Penmaenbach rhyolitic intrusion and the Cader Idris granophyre<sup>2-5</sup> are of Ordovician age, and it has commonly been inferred that the bulk of acid igneous activity in the region is coeval. On the other hand, the view is held by some workers that

Table 1 Fisher statistics of a.f. cleaned remanences of palaeomagnetic sites from Lower Palaeozoic igneous rocks of North Wales

Rock unit	Approximate grid reference	Peak a.f. (mT)	N (sites)	R	k	$\alpha_{95}$	D*	I*	Palaeomagnetic pole			
									Long.	Lat.	dψ	dχ
Penmaenmawr granodiorite	SH 700 760	10–40	12	11.6	27	8	164	–71	104E	81S	13	15
Penmaenbach rhyolitic intrusion	SH 750 780	20	12	11.5	21	10	125	–71	112E	59S	15	17
Foel Fras granodiorite	SH 699 695	20	4†	3.9	214	5	207	–78	209E	71S	9	9
Garnfor granodiorite	SH 360 460	20–50	9	8.7	30	13	136	–62	84E	60S	11	14
Carreg-y-Llam granodiorite	SH 340 440	40–80	5	4.9	37	13	126	–71	111E	59S	19	22
Bodelias granodiorite	SH 320 420	20–60	6	5.8	27	19	120	–55	87E	45S	13	19
Myndd Nefyn granodiorite	SH 320 410	30–80	7	6.5	11	14	139	–36	55E	44S	12	20
Gurn Ddu granodiorite	SH 400 470	20–60	6	5.8	22	9	244	–72	233E	55S	22	25
Yr Eifl microgranite	SH 350 450	20	6†	5.9	62	14	82	–65	122E	32S	15	19
Cader Idris basalts	SH 710 146	20–60	10	9.4	13	14	208	–73	229E	73S	18	22
Overall mean of above results (all sites)			69	63.1	11	5	150	–69	96E	72S	8	9
Overall mean of above results (excluding Myndd Nefyn and Cader Idris)			52	50.0	17	5	143	–71	108E	68S	8	9

\*All mean directions quoted are *in situ* except for that of the Cader Idris basalts which has been tilt-adjusted by simple rotation about the present strike.

†Statistics presented are the within-site result from a single site at Foel Fras and Yr Eifl.

The notation used is as follows:  $R$  is the resultant of  $N$  unit vectors,  $k (= N-1/N-R)$  is the estimate of Fisher's<sup>13</sup> precision  $\kappa$ ,  $\alpha_{95}$  is the semi-angle of the cone of 95% confidence about the mean direction  $D$  (declination in degrees east of north) and  $I$  (inclination from horizontal, positive indicating downwards),  $d\psi$  and  $d\chi$  are the semi-angles subtended by the major and minor axes of the ellipse of 95% confidence about the mean pole position.

some intrusions belong to a late stage of the Caledonian orogeny. In particular, W. E. Tremlett has argued that the granodiorite complex of northern Llyn is associated with NE–SW Caledonoid faults of that area, and is therefore probably of early Devonian age; it is geochemically distinct from the Ordovician acid rocks<sup>7–9</sup>. He has also argued on field evidence that the Penmaenmawr microgranodiorite is post-orogenic, and in disagreement with Davies<sup>6</sup> assigns a similar age to the Foel Fras granodiorite 6 km south of Penmaenmawr (W. E. Tremlett, personal communication). The Penmaenmawr intrusion has given a K–Ar age of 375 Ma<sup>10</sup> from an admittedly weathered sample. The Llyn granodioritic intrusions have yielded Hercynian ages (R. M. McIntyre, personal communication) but there is no geological evidence to suggest massive igneous activity in North Wales at that time, and these apparent radiometric ages are therefore to be regarded as minimum estimates.

Many of the intrusions have been sampled during our new study and the palaeomagnetism of those possessing stable remanences after a.f. cleaning is reported here (Table 1, Fig. 1). Typically at least six independently orientated specimens were obtained at each site. Total NRM's were generally well grouped, but in a few cases large randomly directed magnetically soft components were removed in low alternating fields, enabling a well grouped stable remanence to be isolated. The criterion for optimum magnetic cleaning is that of Briden<sup>14</sup>. In all cases, progressive a.f. demagnetisation experiments have shown the observed remanences to be of high coercivity and in most cases directionally stable to peak alternating fields > 100 mT. Microscopic examination of polished specimens generally shows euhedral-subhedral magnetite grains with lamellar intergrowths of ilmenite, a textural combination associated with high temperature (> 600 °C) petrogenesis<sup>11</sup>. In spite of the unavailability of baked contact tests or other geological evidence for the age and origin of remanence, the stable NRM is therefore likely to be a TRM acquired during initial cooling. With the exception of the results from the Myndd Nefyn granodiorite, which may be deviant due to slight relative movement in an area of undeniably complex faulting<sup>9</sup>, the *in situ* remanences are well grouped with an overall mean of  $D=143^\circ$ ,  $I=-71^\circ$  corresponding to a conventional palaeomagnetic pole at  $108^\circ\text{E}$ ,  $68^\circ\text{S}$ .

This mean pole is nearly  $90^\circ$  away from published Ordovician–Devonian poles for Britain and an explanation must be sought.

Inevitably there are time gaps in the palaeomagnetic record, and it may well be that the new data fall in one of these. Gaps of the order  $10^7$  yr are to be expected given the sparseness of published data. Therefore if the discrepancy is to be explained in terms of polar shift on the assumption of an axial geocentric dipole field, then apparent polar wander at an average rate approaching  $20^\circ \text{ Myr}^{-1}$  has to be invoked. This is an order of magnitude greater than apparent polar wander arising from plate motions in more recent geological times. Even if it were accomplished by minimum lateral motion (given by the palaeolatitude change) together with rotation about a vertical axis close to the sampling area (to accommodate the declination discrepancy), motion of  $> 50 \text{ cm yr}^{-1}$  is required, which is still excessive on any plate tectonic analogy.

An interval of  $10^7$  yr might also be thought reasonable for the emplacement of these widely spaced North Wales bodies. It is hard to imagine an overall intrusion time of  $< 10^6$  yr for such a province. Hence the possibility that these rocks record a single geomagnetic polarity transition is effectively excluded because estimates on Tertiary to Recent transition times indicate that they typically take  $10^3$ – $10^4$  yr. Nevertheless it is intriguing to note that the pole position from the North Wales studies is almost equatorial to the typical Ordovician palaeomagnetic field. This is in common with the finding of Shaw<sup>12</sup> that the field paused in this half-way state during a polarity transition recorded in the palaeomagnetism of some Icelandic rocks.

In an area of such complex structural history as North Wales it might be expected that the *in situ* remanences of the intrusions require adjustment for tilt and/or local rotation. This does not, however, seem to be the case, as these massive intrusions, many of which have near vertical 'stock-like' shapes, would require an unlikely  $60^\circ$  northward rotation about a horizontal axis to remove the palaeomagnetic discrepancy. Also, they are seen to carry near vertical contemporaneous dikes. Table 1 and Fig. 1 also contain the tilt-adjusted palaeomagnetic results from the structurally simple Ordovician Upper and Lower Basic Lavas of Cader Idris. Their overall mean magnetisation falls within the group of *in situ* results from the intrusions. The magnetic stability of the lavas was comparatively low, but the similarity of the two results does reinforce the suggestion that the intrusions have suffered little tectonism. Therefore unless evidence is produced to show that the bodies sampled in this study, which are geographically

widely scattered from Cader Idris to Conway, have all been rotated by the same amounts by a bodily movement of North Wales, then their *in situ* remanences must be considered to define the geomagnetic field at the time when those remanences were acquired.

In North Wales there is no geological evidence of any large scale post-Caledonian thermal event at high enough temperatures to explain the stable remanent magnetisations observed in this study. In any case the only known palaeomagnetic field corresponding to these results is that of Tertiary to Recent times. The small amount of Tertiary igneous activity in North Wales could not have been responsible for magnetising rocks on the large scale necessary to explain the observations.

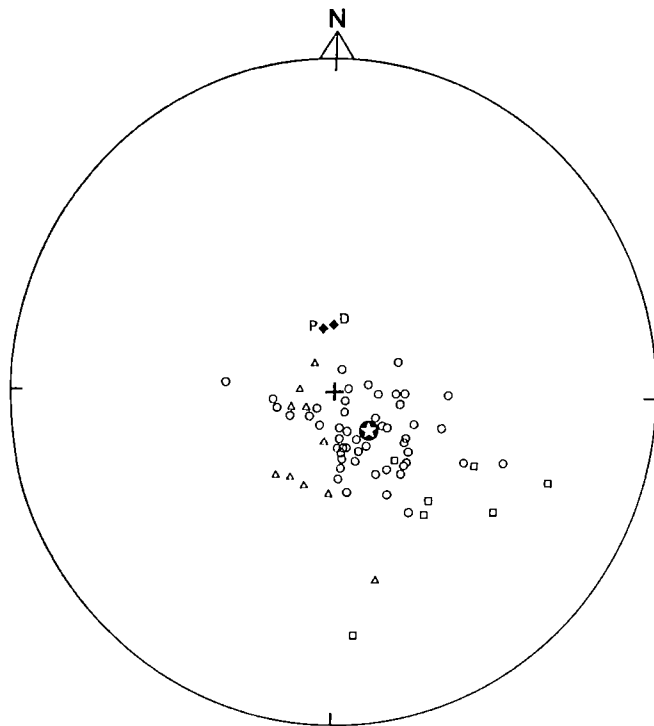


Fig. 1 Stereographic projection of site mean remanences after a.f. cleaning of intrusives from North Wales, and the basic lavas of Cader Idris. O, *In situ* remanences in all intrusive rocks except Myndd Nefyn; the star indicates their overall mean direction; □, the *in situ* results from Myndd Nefyn; Δ, tilt-adjusted results from the basic lavas of Cader Idris. The present axial geocentric dipole and the present geomagnetic field directions are marked ♦ and labelled D and P respectively. Open symbols indicate negative (upwards) inclinations and closed symbols positive (downwards) inclinations.

All conventional interpretations of the results of this study are therefore unsatisfactory, and instead, the possibility of an anomalous geomagnetic field lasting several million years during the Lower Palaeozoic must be seriously considered. It is most likely that we have recorded only one such anomalous event in this study because of the improbability of successive intrusions repeatedly occurring at similar geomagnetically anomalous instants. Evidence of precisely when this anomaly might have existed is inconclusive. Its occurrence in late Ordovician times would, however, be compatible with the inferred primary TRM of the intrusions, their possible Ordovician age and the similarity of their remanence directions with that of the proven Ordovician Cader Idris lavas.

The reality of an anomalous magnetic field lasting  $\sim 10^7$  yr would be of considerable geomagnetic interest. Whether

it exists or not should be capable of resolution by palaeomagnetic studies elsewhere in the world.

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## Palaeomagnetism of a slowly cooled plutonic terrain in western Greenland

PALAEOMAGNETIC results from a high grade Precambrian terrain in western Greenland show many features attributable to magnetisation during very slow cooling caused by gradual uplift and erosion. They may locate a whole segment of the Laurentian apparent polar wander (APW) path with unusually high precision and also indicate the probable direction of movement.

The magnetic effects described here are analogous to those experienced by radiometric systems during slow cooling<sup>1,2</sup>, and are attributable in part to the fact that magnetic minerals have a range of blocking temperatures at which the magnetisation becomes effectively fixed. The effective blocking temperatures of magnetic grains will be considerably lowered during slow cooling, possibly to as low as 200 °C (refs 4 and 5) so that magnetisation would be acquired during the later stages of unroofing, when moderate erosion rates can be expected. Average present-day erosion rates have been variously estimated at 30 and 76 m Myr<sup>-1</sup> (refs 6 and 7, respectively), and if a slightly higher rate of 100 m Myr<sup>-1</sup> is assumed together with a geothermal gradient of 20 °C km<sup>-1</sup>, and a blocking temperature range of 50 °C for stably magnetised grains, then the magnetisation of any one specimen would take 25 Myr. Also, because the magnetisation will be acquired as the rocks pass through the (presumably horizontal) isotherms corresponding to their critical range of blocking temperatures, rocks at higher structural levels will become magnetised earlier than those at lower levels. For example, with an erosion rate of 100 m Myr<sup>-1</sup>, a difference in level of 2 km would correspond to a difference in the mean time of magnetisation of the order of 20 Myr.

Slow cooling during unroofing should therefore produce several effects in the palaeomagnetic record.

- Apart from the effects of APW all of the rocks formed before uplift should be magnetised in the same direction and give the same pole position, even though geological evidence indicates that they are of different ages.

- Because secular variation should effectively be averaged out within each specimen, between-site scatter attributable to this cause should be small.

- If APW occurred during the magnetisation period then poles from sampling sites at different original structural levels should be strung out along a trend corresponding to that particular section of the APW path, with higher sites at the older part of the trend and lower sites at the younger part.

● If they have the same mean blocking temperature different sampling sites at the same structural level should give poles close together. Any variation in mean blocking temperature, however, will clearly affect the time of magnetisation, and so poles from sites with high blocking temperatures should be displaced towards the older part of the trend compared with sites with lower blocking temperatures at the same level. Because of this it is unlikely that there will be a perfect relationship between the position of a pole in the trend of poles and the structural level of the corresponding sampling site. Determination of mean blocking temperatures in the laboratory should confirm the sense of time along the trend indicated by comparing the position of poles from sites at different structural levels.

● As any specimen has probably taken millions of years to cool through its critical range of blocking temperatures it should contain the magnetic record of that interval of time. Progressive thermal demagnetisation should therefore effectively move the magnetisation of a specimen from the lowest to the highest blocking temperature direction. Two possibilities arise. First, if the blocking temperature spectrum has two peaks corresponding, for example, to haematite and magnetite, then two distinct magnetisations may be present, an older one corresponding to haematite and a younger one corresponding to magnetite. Progressive demagnetisation would cause the magnetisation to move towards the older direction, but the actual movement would simply follow the great circle connecting the two directions. Such results have been reported from the Grenville province<sup>14</sup>. Secondly, if there is a continuous spectrum of blocking temperatures attributable, for example, to a continuous variation in grain size or ionic substitution, then a continuous spread of directions of magnetisation should exist between those corresponding to the highest and lowest blocking temperatures. Progressive demagnetisation would again cause the magnetisation to move towards the older direction, and the actual movement should indicate, after suitable vector analysis, the form of that particular section of the APW path.

● If rocks cool through their critical range of blocking temperatures during a period when the Earth's field is reversing itself at the same rate as at present they would be effectively demagnetised<sup>8</sup>. For rocks to acquire any magnetisation they must cool either during a prolonged period of constant polarity, or in a period when one polarity is dominant. Thus in a slowly cooled terrain one would expect either that the rocks should carry no stable magnetisation, or that they should all be magnetised with the same polarity.

The results reported here are from an area about 20 km square near Itivdlek some 40 km south of Holsteinsborg in western Greenland. The area is just within the early Proterozoic, Nagssugtoqidian Mobile Belt, close to its gradational boundary<sup>9</sup> with the Archaean craton to the south. The terrain comprises granulite and amphibolite facies gneisses cut by dolerite dykes which vary from fresh to almost completely autometamorphosed but which are essentially undeformed. There is strong geological evidence<sup>10</sup> that the area was at metamorphic temperatures when the dykes were intruded.

Stable magnetisation was found in both dykes (18 sites) and gneisses (4 sites), and after suitable alternating field (a.f.) demagnetisation most sites showed an unusually low within-site scatter of directions. Fourteen sites had a precision parameter,  $k$ , of better than 300 (corresponding to a circle of 95% confidence with a radius  $\alpha_{95}$  of less than  $3.2^\circ$ ), and at no site was  $k$  less than 50 ( $\alpha_{95}=7.9^\circ$ ). Largely because of the accuracy with which the site poles are determined the effects of slow cooling can be recognised.

Figure 1 shows that 18 poles (15 dykes and 3 gneisses) are arranged in a linear trend approximately  $10^\circ$  wide and

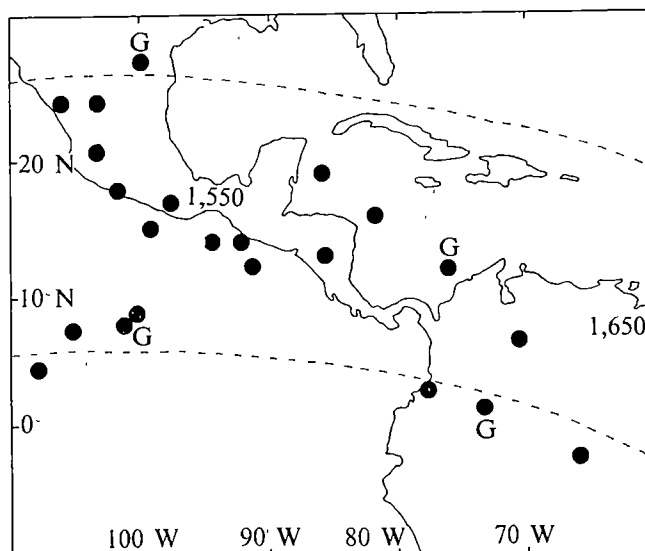


Fig. 1 The 22 site poles. Each is the mean of, generally, eight separately oriented specimens. The 18 dyke poles are unlabelled; G, the four gneiss poles. The poles have been rotated to correct for the drift of Greenland away from North America<sup>11</sup> to allow comparison with the appropriate part of the latest published APW path<sup>12</sup> for Laurentia (dashed band, with assigned ages in Myr). Most site poles initially showed southerly movements with progressive a.f. demagnetisation, presumably due to the removal of recently acquired viscous components, and at 7 sites (see text and Fig. 3) these were followed by systematic south-easterly movements. The pole selected for each site was generally either at the end point of the southerly movement or at the point where the southerly movement changed to a south-easterly movement, and was usually at a higher demagnetising field than that which gave the best within-site precision.

$45^\circ$  long, running NW-SE, and making a slight angle with the appropriate portion of the latest Laurentian APW path<sup>12</sup>. The remaining 4 poles are grouped together just to the south-west of the trend, which cannot be explained at present. All 174 specimens examined were magnetised with the same polarity. After correcting for the drift of Greenland away from North America<sup>11</sup> the mean direction of magnetisation for all 22 sites gives a pole at  $13^\circ\text{N } 91^\circ\text{W}$ , with  $k=47.1$  and  $\alpha_{95}$  of  $4.6^\circ$ .

All of the sampling sites were within 50 m of sea level, so if the linear trend of poles is attributable to differences in the original structural level there must have been a slight tilting of the area subsequent to magnetisation. For example, a tilt of  $5^\circ$ , which would be difficult to detect geologically in such a terrain, would be equivalent over a horizontal distance of 20 km to a change in the original level of 1.8 km. This possibility is supported in this case by a general, though imperfect, relationship between the position of a pole in the linear trend of poles and the geographical position of the corresponding sampling site (Fig. 2): most of the sites in the NNW part of the area give poles in the south-eastern part of the trend, and sites in the SSE part of the area give poles in the north-western part of the trend. If the north-western part of the trend is the younger, the results suggest that the SSE part of the area represents the lowest structural level, that is, that there has been tilting of the terrain towards the NNW. The poles from sites D16, D17 and G20 (Fig. 2) clearly do not conform to this pattern, those sites are the most northerly in the area and so their poles should plot at the south-eastern and not the north-western end of the trend. But a prominent E-W fault, for which there is some geological evidence that the movement has been predominantly vertical (D. Nash, personal communication), separates them from the remainder of the sampling area. The apparent displacement of the anomalous poles could thus be caused by the area north of the fault having moved up with respect to the area to the south.



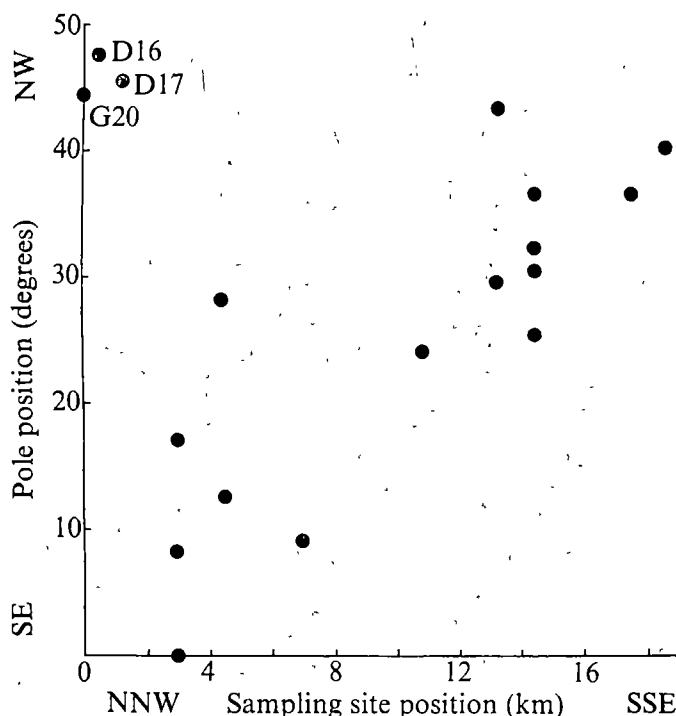


Fig. 2 The relationship between sampling site positions and corresponding pole positions for the 18 poles in the linear trend. Ordinate, the angular distance north-west along the linear trend to each pole from the D13 pole (the most south-easterly pole); abscissa, distance SSE to each sampling site from an imaginary ENE trending line through site G20 (the most northerly site).

If the linear trend of poles is due to APW during slow magnetisation then the scatter of poles attributable to other causes, represented by the  $10^\circ$  width of the trend, is equivalent to an angular standard deviation of about  $4^\circ$ . This is much less than the values of  $9\text{--}24^\circ$  quoted for secular variation for the appropriate palaeolatitude<sup>13</sup> indicating either that magnetisation was extremely fast, which seems inconceivable on geological grounds and would not in any case explain the linear trend, or that it was so slow that secular variation was effectively averaged out.

During progressive a.f. demagnetisation the site mean pole at seven sites showed small but very systematic movements always in a south-easterly direction (Fig. 3). As there is probably a reasonably close relationship between coercive force and blocking temperature, the systematic movements may represent the movement of the magnetisation from a younger direction, corresponding to grains with lower blocking temperatures, to an older one attributable to grains with higher blocking temperatures. Vector analysis of the movements indicates that, in general, the component actually removed in each step moves progressively in a south-easterly direction, suggesting either that there are two distinct magnetisations with overlapping coercivity spectra, or that there is a continuous spread of magnetisations. The latter seems more likely because the average direction of systematic movement is close to the trend of the poles, indicating that both record the APW path over that period of time. The linear trend of the poles is not greatly different in direction from the trend of the latest published APW path at that point<sup>12</sup>, and the sense of time along the trend of poles as indicated by the systematic movements (younger towards the north-west and older towards the south-east) is in accord with the age dates assigned to the APW path.

Thermal demagnetisation studies were carried out on six specimens from sites which all showed systematic south-easterly movements during a.f. demagnetisation. Up to  $520^\circ\text{C}$  the mean pole moved generally southwards but

between  $520$  and  $560^\circ\text{C}$ , when by far the major decrease in the intensity of magnetisation occurred, the mean pole moved systematically in a south-easterly direction very close to the mean direction of the systematic a.f. movements (Fig. 3). It therefore seems that the stable magnetisation of the specimens resides in grains of magnetite or titanomagnetite which have blocking temperatures (measured on the laboratory time scale) of between  $520$  and  $560^\circ\text{C}$ , and that the systematic south-easterly movements also occur over that interval.

According to the assigned ages on the latest APW path the trend of poles covers an age range from about  $1,525$  to  $1,650$  Myr BP, but an examination of the well dated poles in the area suggests that this portion of the APW path may be about  $100$  Myr older than indicated. Ages are not yet available from the sampling area, but regional K-Ar mineral ages from the Nagssugtoqidian Belt as a whole range from  $1,650$  to  $1,790$  Myr BP, and provisional U-Pb studies on zircons give ages of  $2,200\text{--}2,600$  Myr BP (ref. 14). There thus seems to be good agreement between the time of magnetisation and the setting of the K-Ar mineral clocks, indicating that the magnetisation was probably acquired during a fairly late stage of uplift and cooling long after the peak of metamorphism.

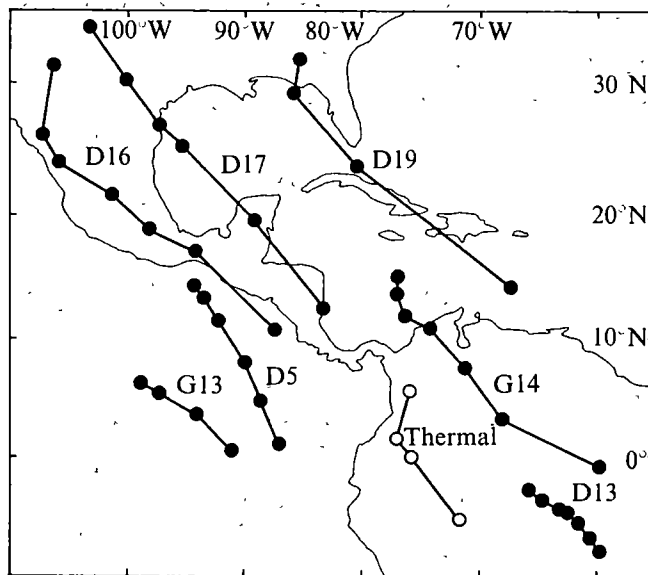


Fig. 3 Systematic south-easterly movements of the mean pole with progressive a.f. demagnetisation at seven sites. For several sites the final part of the southerly movement is also shown to illustrate the transition from southerly to south-easterly movement. ○, The movement of the mean pole of six selected specimens with progressive thermal demagnetisation between  $500$  and  $560^\circ\text{C}$ . All of the poles have been corrected for the drift of Greenland as in Fig. 1. The poles for D17 and D19 have also been moved  $10^\circ$  north, and the thermal poles  $9^\circ$  south-west, to improve the clarity of the diagram. The demagnetising fields (oersted) were—D5: 200, 300, 400, 600, 800, 1,000; D13: 150, 200, 300, 400, 600, 800, 1,000; D16: 0, 50, 100, 200, 300, 400, 600; D17: 50, 100, 150, 200, 400, 600; D19: 100, 150, 200, 400; G13: 600, 800, 1,000, 1,200; G14: 100, 150, 200, 400, 600, 800, 1,000. For thermal demagnetisation the temperatures were  $500$ ,  $520$ ,  $540$  and  $560^\circ\text{C}$ .

The results thus seem to show all of the characteristics to be expected from a slowly cooled terrain. If results of this type prove to be common in plutonic terrains they will not only provide valuable details about apparent polar wander, but could also be a useful tool for investigating movements, such as tilting and faulting, which have occurred subsequent to magnetisation, and which often cannot easily be studied by other methods.

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## Effect of depth of burial and tectonic activity on coalification

THE study of variations in coal rank is important to coal geologists and the coal industry, and a proper understanding of the coalification process is also important to oil exploration geologists. The rank of coals, which occur as thin seams or lenses in oilfield sediments, can be regarded as an indicator for the degree of maturation of the organic matter (kerogen), finely dispersed in the sediments. Coal and kerogen are somewhat similar in chemical composition, and so are controlled by the same physical and chemical factors. If tectonic pressure can be shown to advance coalification, then it follows that a higher maturation of kerogen can be expected near a fault zone.

Coalification occurs when original plant material becomes buried, and gradual changes in its chemical composition and physical properties take place. The changes can lead to the formation of lignite, brown coal, bituminous coal or anthracite. During the late nineteenth century, it was widely supposed that burial pressure on coal was responsible for the advancement of coalification. In some tectonically disturbed coalfields, for instance, the presence of high rank coals were found which led geologists to believe that tectonic pressures accelerated coalification<sup>1</sup>. Other studies in coalfield geology and coal chemistry have convincingly shown that the role of pressure is insignificant in the process of coalification<sup>2–6</sup>, and the results of experiments simulating coalification<sup>6</sup> indicate that the lower the pressure, the more rapid the coalification at a given temperature. From thermodynamics it is known that the gross reaction in chemical processes, including coalification, is determined mainly by temperature and time, and that static pressure retards reactions because it restricts the removal of reaction products<sup>7</sup>. A coalification pattern which does not seem to fit these studies has been reported for the Bowen Basin of Queensland, Australia (see ref. 7; and Fig. 1). We believe, however, that the pattern in this area is attributable to differences in the original depths of burial below subsequently eroded sediments.

During the Palaeozoic, a sediment-filled, linear downwarp formed along the eastern margin of Australia, separating an old land mass to the west from a rising orogenic belt to the east. During the Permian, coal-bearing formations were deposited; they were followed by as much as 5 km

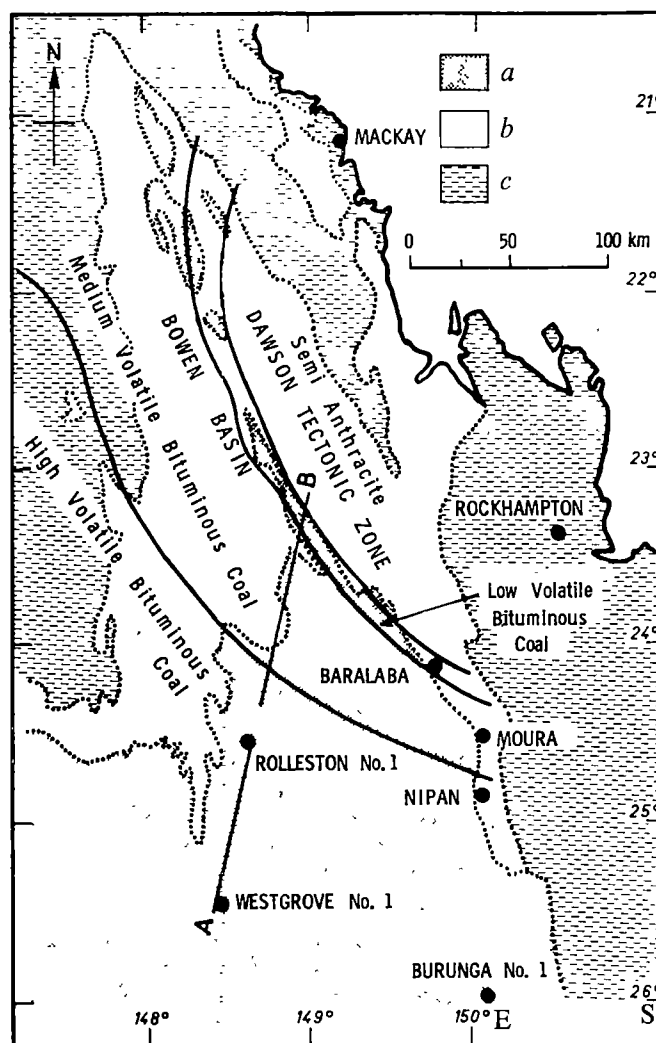


Fig. 1 Coalification trends in the Bowen Basin (after ref. 7). A–B, Location of cross section shown in Fig. 2. a, Mesozoic sediments; b, Permian sediments; c, pre-Permian sediments.

of Triassic sediments. After the deposition of these sediments, the area, particularly the northern part, became uplifted and suffered intense erosion. Contemporaneously, a large synclinal structure formed along the downwarp, the northern part of which is now the Bowen Basin. Tectonic activity was particularly strong along the eastern flank of the Bowen Basin, and the so-called Dawson Tectonic Zone formed. In areas close to this zone, semi-anthracite coals are now exposed at the surface. With increasing distance from this zone towards the south-west, the rank of outcropping coal seems to decrease regularly, down to that of high volatile bituminous coals over a distance of 100 km. This coalification pattern may have been generated by the formation of the Dawson Tectonic Zone (see, for example, ref. 7). Possibly, the tectonic activity together with the deep burial of the coal seams may have been responsible for the increase in rank<sup>8</sup>, though pressure tends to inhibit rather than promote increase in rank<sup>9</sup>. Shibaoka *et al.*<sup>10</sup>, however, have shown that temperature increases attributable to deep burial were probably responsible for the increase in rank around the Dawson Tectonic Zone.

No evidence has been given to explain why the degree of tectonic disturbance gradually and regularly decreases away from the Dawson Tectonic Zone—only the zone itself is heavily disturbed. Even if tectonic pressure did decrease regularly over 200 km, it would not affect the coalification over such a distance<sup>2–6</sup>. The only real effect that the

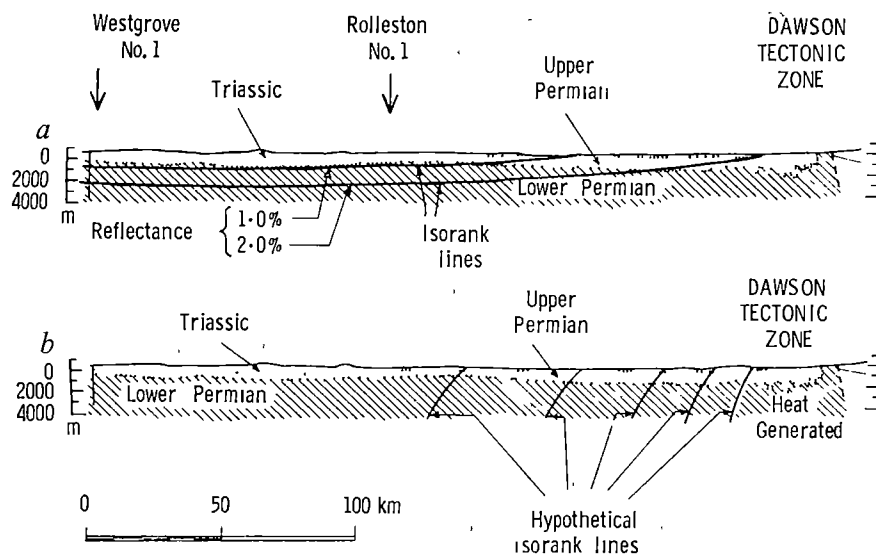
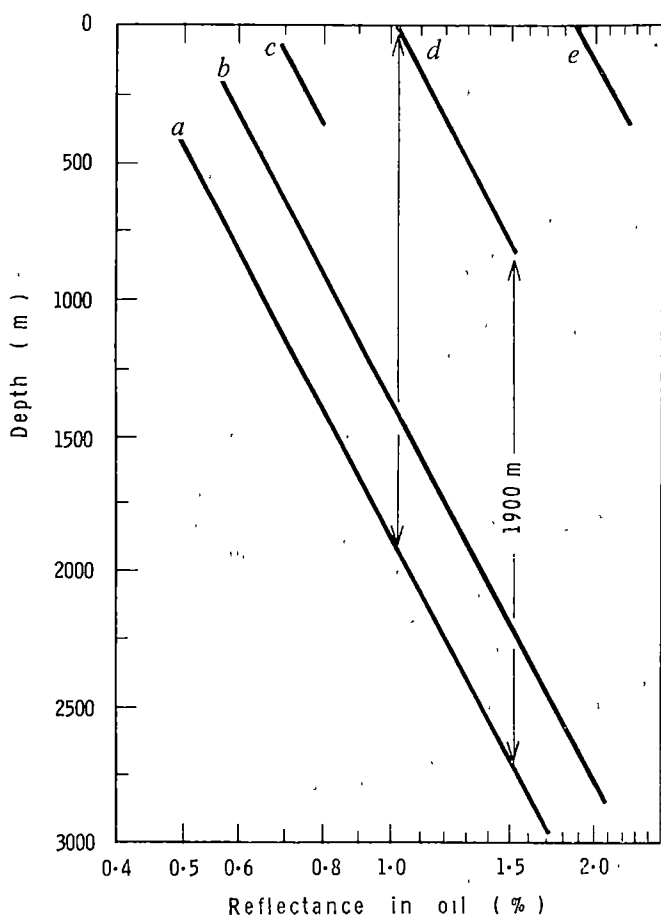


Fig. 2 Reflectance profiles from Westgrove to the Dawson Tectonic Zone showing two alternative models for the isorank lines: a, isorank lines controlled by depth of burial followed by uplift and erosion of the western part of the area; b, isorank lines drawn assuming a heat source associated with the Dawson fracture.

Dawson Tectonic Zone could have had on coalification must have been attributable to the heat generated during the tectonic activity.

If the zone formed within the space of a few years, which is questionable, the heat generated could possibly have been sufficient to have heated the coal-bearing formations up to at least 200 °C. In that case the isorank planes would incline steeply (Fig. 2b) unless the heat source was extremely deep. We have measured the reflectance of coals in boreholes from this area to construct isorank planes. The planes are inclined at an angle of about 1° 30' (3 km in 110 km) between Rolleston and the Dawson Tectonic

Fig. 3 Depth-reflectance curves for coal bores and oil wells in the southern part of the Bowen Basin: a, Burunga No. 1; b, Rolleston No. 1; c, Nippan; d, Moura; e, Baralaba.



Zone (Fig. 2a), thus requiring a local heat source at least 40 km deep. It therefore seems difficult to explain how the geographical variation of coal rank can be attributed to heat generated along the Dawson Tectonic Zone.

A more likely cause of a gradual increase of temperature towards the Dawson Tectonic Zone could have been a thickening of Triassic overburden which has subsequently been lost by erosion. During the Triassic an old land mass lay to the west, and Triassic sediments became progressively thicker towards the depositional centre of the trough which was probably near the present axis of the Bowen Basin.

The Dawson Tectonic Zone may have coincided<sup>11,12</sup> with the zone of maximum deposition during the Permian and the Triassic. Geological mapping indicates that originally there could very well have been as much as 3 km of Triassic sediments in the central and northern parts of the Bowen Basin. Depth reflectance curves for coal samples from various oil wells suggest that the palaeogeothermal gradient was much the same over the whole southern part of the Bowen Basin, and that where high rank coal is now at a shallow depth, a thick overburden would have been lost by erosion. At Moura, for example, an extra 1,900 m of Triassic sediments could have been lost, compared with a minimal loss at Burunga (Fig. 3). It also seems that a reverse trend may have followed the termination of the downwarp.

We believe that the increase in coal rank in the Bowen Basin was caused neither by tectonic pressure acting on coal seams, nor by the heat generated by the tectonic activity of the Dawson Tectonic Zone. We believe that the main cause was a normal geothermal gradient in thick sediments.

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## Desert varnish and marine ferromanganese oxide nodules: congeneric phenomena

BLACK, hydrated ferromanganese oxide (FMO) concretions of microscopic to macroscopic size and complex mineralogy cover ocean floors all over the world at depths of up to 8 km (ref. 1). These amorphous-crystalline nodules have a total Cu, Ni and Co content of 40 p.p.m. to 4% and vary greatly in trace element content, apparently because of varying growth rates caused by the different *Eh* and *pH* in their environments. Black, amorphous, hydrated desert varnish (DV) coatings, 10–100  $\mu\text{m}$  thick, are extensively found in limited areas of the world's arid lands<sup>2,3</sup> in both the subtropics and in non-sterile areas of the Antarctic dry valleys<sup>4</sup>. Marine FMO nodules are formed principally by biological fixation processes; the mode and rate of formation of DV, on the other hand, are not understood, but concern anthropologists in their efforts to date petroglyphs and other artefacts of ancient man<sup>5</sup>.

The broad similarities in element enrichment shared by FMO forms from terrestrial and marine deserts<sup>6</sup> (Fig. 1) indicate a shared formation process<sup>7</sup>. Here, similarities between the natural FMO forms are compared to postulate a formation process for DV. This process seems to be started geochemically but later to involve the cation-scavenging properties of colloidal, hydrated  $\text{MnO}_2$  (ref. 8) as affected by chemolithotrophic bacteria<sup>9–11</sup>. All natural FMO forms seem to show broad similarities in elemental composition and nucleation, which should logically lead to a similar mineralogy on subsequent crystallisation, if this occurs.

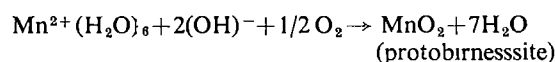
Figure 2 shows the 'genetic classification' diagram of Bonatti, Kraemer and Rydell<sup>12</sup> for lake and marine nodules. Hydrogenous deposits (the group *a*, *b* and *c*) seem to grow extremely slowly, by the precipitation of Fe and Mn from normal seawater in oxidising conditions. In contrast, hydrothermal deposits associated with geothermal activity (group *d*, *e* and *f*) grow quickly, whereas the diagenetic group (*g*, *h* and *i*) forms at varying rates through the remobilisation of Mn from sediments in reducing conditions. Halmyrolytic deposits (not shown) result from the submarine weathering of basalt.

In general, the slowly accreted nodules have high Th/U ratios, transition and trace element levels and degrees of

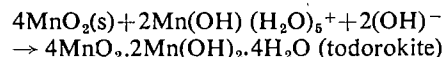
crystallinity. Nodular Ce/La ratios and rare earth element (REE) contents are also inversely proportional to accretion rates<sup>13</sup>, whereas low Mn/Fe ratios and REE depletion are connected with rapid growth in shallow continental margins. The low Co–Ni–Cu content of varnishes places them in the rapidly formed hydrothermal group, *d*, *e* and *f* in Fig. 2. Although Ce has not yet been reported in varnishes, their La/Yb ratio approximates that of Glasby's<sup>13</sup> 'fast accreted' nodules from Aden and Loch Fyne. Desert varnish has formed in North African and Californian deserts within a period of 25–50 yr (refs 2 and 14).

Burns and Brown<sup>15</sup> have shown by microprobe transection that Fe deposition precedes that of Mn in the marine environment, thus ' $\text{MnO}_2$ ' on coral shows an increasing relative concentration of Mn towards surface. This is also the case for DV<sup>16</sup>, with Mn concentration increasing outwards from the leached rock surface as the Fe, Ca, Mg, Ti, Si, Al and K levels decrease. The initial deposition of  $\text{Fe}^{3+}$  has been attributed<sup>15</sup> to precipitation of  $\text{FeOOH} \cdot n\text{H}_2\text{O}$  at local high *pH/Eh* nucleating sites, where weathering has left concentrations of phosphate, silicate or carbonate; precipitation of negatively charged colloidal  $\text{Mn}^{2+}$  hydroxide, aided by the opposing zero charge potentials of the two hydroxides follows.

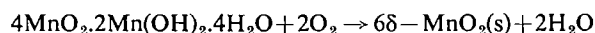
It has been suggested<sup>15,17</sup> that following nucleation,  $\text{Mn}^{2+}$  in a high *pH/Eh* marine environment reacts as follows: first, initial deposition



Second, adsorption of more  $\text{Mn}^{2+}$



Third, oxidation of todorokite to form  $\delta - \text{MnO}_2$

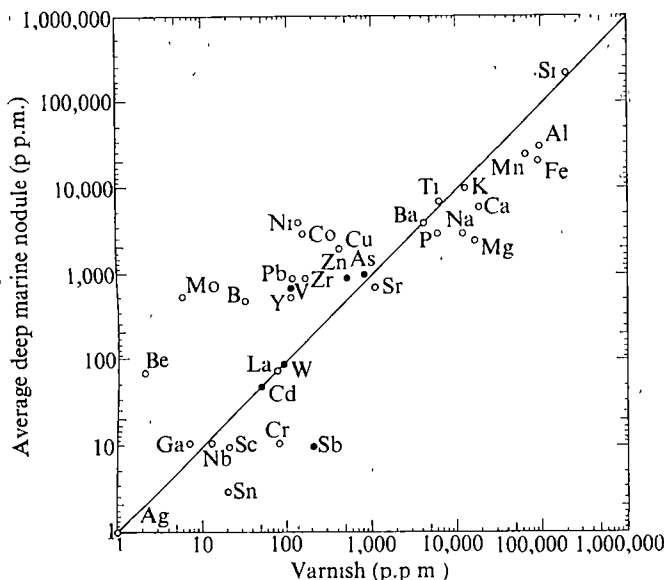


For deep nodules this process eventually leads to the single authigenic high pressure-stable crystalline phase, todorokite, which readily oxidises and dehydrates to yield minute platelets of  $\delta - \text{MnO}_2$  admixed with a separate exsolved  $\text{FeOOH} \cdot n\text{H}_2\text{O}$  phase. Detrital particles with (–) zero electric potentials, such as quartz, are excluded from the growing nodular surface and are also absent from DV.

Subsequent crystallisation occurs slowly, if at all, with the epitaxial intergrowth of species such as hollandite  $(\text{Ba}, \text{K}, \text{Pb}, \text{Na})_{1-2} \text{Mn}_8\text{O}_{16} \cdot n\text{H}_2\text{O}$ , psilomelane  $(\text{Ba}, \text{K}, \text{Mn}, \text{Co})_2 \text{Mn}_5\text{O}_{10}$ ,  $n\text{H}_2\text{O}$ , birnessite  $(\text{Ca}, \text{Na}, \text{Mn})_x \text{Mn}_7\text{O}_{14} \cdot 3\text{H}_2\text{O}$  and todorokite  $(\text{Na}, \text{Ca}, \text{Mn})\text{Mn}_3\text{O}_7 \cdot n\text{H}_2\text{O}$ , intergrown with polymeric ferrihydrite,  $\text{FeOOH} \cdot n\text{H}_2\text{O}$ .

These associations of elements suggest (Fig. 1) that the apparently amorphous dioctahedral 2:1 sheet silicate 'lattice' of DV probably contains cryptocrystalline microdomain manganese mineral phases as do the nodules, particularly as Ba appears in all reported DV. 'Goethite' has been reported<sup>14</sup> in one X-ray study of DV, and this work should be extended to obtain a comparison with the known line spectra of the marine nodular phases. These mineral and trace element suites have also been found in Australian desert soil microconcretions<sup>18</sup>. Rectilinear semi-log plots of DV Mn against Ba, Co, B, Sn, Y and Yb<sup>2</sup> have been obtained in partial agreement with ref. 12, where the Mn correlation to Ba and Co in marine nodules is shown. The DV data of Lakin *et al.*<sup>3</sup> though scattered, show a similar Mn–Ba–Co correlation. Colloidal scavenging of trace cations is probably involved in the formation both of DV and marine nodules. Murray<sup>19</sup> has shown that precipitated hydrated  $\text{MnO}_2$  adsorbs  $\text{Co} > \text{Mn} > \text{Ni} > \text{Ba} > \text{Sr} > \text{Ca} > \text{Mg}$ , with Co irreversibly sorbed at an alkaline *pH*, whereas manganite sorbs Ni, Ca, Zn, Mg and  $\text{Ti}^{4+}$  by lattice replacement.

Fig. 1 Average elemental abundance in deep-sea FMO nodules<sup>6</sup> relative to average desert varnishes (cited) ○, Taken from ref. 2. Averages for Stoddard rhyolite fan, Halloran andesite colluvial, and Sheephead andesite colluvial. ●, From ref. 3. Averages of DV3, 5 and 6.





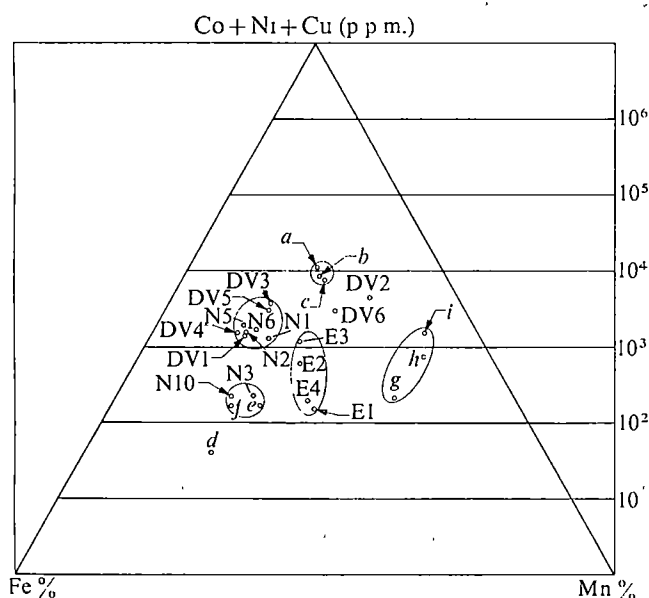


Fig. 2 Ratio Fe:Mn:(Co+Ni+Cu) of representative desert varnishes and hydrothermal and diagenetic marine and lake FMO deposits. Groups a, b and c = hydrothermal; d, e and f = hydrothermal; g, h and i = diagenetic (data of ref. 12). Desert varnishes: DV1-6 and N1, 2, 3, 5 and 6 from ref. 3; E1, Stoddard rhyolite; E2, Halloran andesite; E3, sheephead andesite; E4, Coxcomb tuff of Engel and Sharp<sup>2</sup>.

As noted, nucleation in DV is similar to that in marine nodules but occurs in the alkaline, weathered rock surface where there are silicate, phosphate or carbonate salts. Hem<sup>20</sup> has shown that feldspar increases the rate of oxidation of Mn<sup>2+</sup> to Mn<sup>4+</sup>, and such phenocrysts may initially be more varnished than the matrix. They may, however, weather more rapidly than the matrix and lose their DV, as is the case for limestone. Dewfall (a major water source<sup>21</sup> and weathering agent in arid lands) is the likeliest source of the proper amount of moisture to form DV, which is soluble in rain<sup>5,14</sup>. Other 'constants' in the desert environment are a saline dustfall and an influx of chemolithotrophic microorganisms<sup>22</sup>. Airborne Egyptian desert dust (~20 µm diameter) contained 18% water solubles, chiefly gypsum and salt<sup>23</sup> whereas airborne Colorado Desert dust was also high in salt, gypsum, lead and iron, with small amounts of Zn and Mn. These conditions are necessary and sufficient for the growth of the ubiquitous desert algal microflora.

Following nucleation in the pre-dawn dewfall, biological growth then takes place during the warming and evaporating cycle. This activity is greatest just below the desert surface and would account for the thick 'groundline'<sup>2</sup> of varnish found on cobbles in pavement. The groundline band extends from 1 cm above to 3 cm below the soil surface. An exaggerated 'equatorial band' also develops on certain marine nodules at the sediment-water interface<sup>24</sup>, and this is probably a direct homologue of the DV structure. If desert pavement cobbles are turned over, their unvarnished red undersides become varnished in time, a phenomenon which strongly suggests the phototactic migration of thermophilic motile, inducible<sup>10</sup> Mn reducers and oxidisers (bacteria and fungi) along the rock surface, probably endosymbiotic with the ubiquitous cyanophytes of desert soils. Stones not in contact with the ground are most heavily DV patinated on upper surfaces and in depressions, which would provide favourable nutrient biological niches based on dustfall. This growth mechanism, and the variables involved, is an extension of that proposed by Scheffer *et al.*<sup>14</sup>. As rocks become varnished their blackbody radiation efficiency will also increase thus leading to a thicker DV coating on smaller rocks than on larger ones which cool more slowly. White inert stones such as bull quartz in pavements lack varnish, relative to their neighbours, because they have neither good blackbody radiation

characteristics nor weathering rugosities which trap nutrient dust. Varnishes should be examined for REE anomalies, such as Ce/La, which may apply to their growth rate and age; and they should be examined by means of scanning electron techniques for the surface presence of the admittedly hypothetical bacterial Mn oxidisers.

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## Viscosity in the phase transition region of triblock copolymer systems

THE behaviour of melts of styrene-butadiene-styrene (SBS) triblock copolymer under oscillatory shear exhibits a marked departure from that of homopolymers in the low frequency range; the dynamic viscosity  $\eta'(\omega)$  increases continually with decreasing frequency, rather than exhibiting the Newtonian limiting value  $\eta_0$  expected of true fluids<sup>1</sup>. This peculiar behaviour has been explained in terms of phase separation which forms a lattice of linked polystyrene domains. Here we present rheological evidence directly demonstrating a thermodynamic transition.

We have studied  $\eta'$  at different temperatures for an SBS block copolymer (Shell TR-41-1467; molecular weight 9,600-47,500-9,400) plasticised to different degrees with dipentene ( $T_{\text{hom}}=178^\circ\text{C}$ ), a good solvent for both block types. All experiments were carried out on a Weissenberg Rheogoniometer Model R-17, using a platen diameter of 2.5 cm and a  $4^\circ$  cone angle. Readout of amplitude ratio and phase angle was taken from a strip chart recorder at low  $\omega$  and from Lissajou figures photographed from an oscilloscope at high  $\omega$ . Temperature control was achieved by means of an electric oven enclosing the platens. Small containers of solvent were placed in the oven to prevent solvent loss by the sample. Oscillation amplitudes were chosen to satisfy the conditions of linear viscoelasticity.

We observed that moderately plasticised systems exhibit a limiting viscosity,  $\eta_0$ , at low frequencies if the temperature is raised sufficiently but not so high to cause degrada-

tion of the material (a common problem when working with unplasticised polymer). Results for a system with approximately 30% dipentene are shown in Fig. 1. At 25 and 51 °C the low- $\omega$  anomaly is seen clearly, as reported earlier for the bulk polymer<sup>1</sup> at higher temperatures, but at 76 °C a Newtonian viscosity is recovered. This transition has never been reported for melts, presumably because degradation problems discouraged work at sufficiently high temperatures.

This change in viscosity behaviour can be explained in terms of the concept of 'separation temperature' ( $T_s$ ), as proposed by Leary and Williams<sup>2</sup>. At temperatures below  $T_s$  the domain system is thermodynamically the more stable state, but above  $T_s$  the more stable state is a randomly mixed system behaving as an ordinary liquid with measurable  $\eta$ . The results shown in Fig. 1 would indicate that for

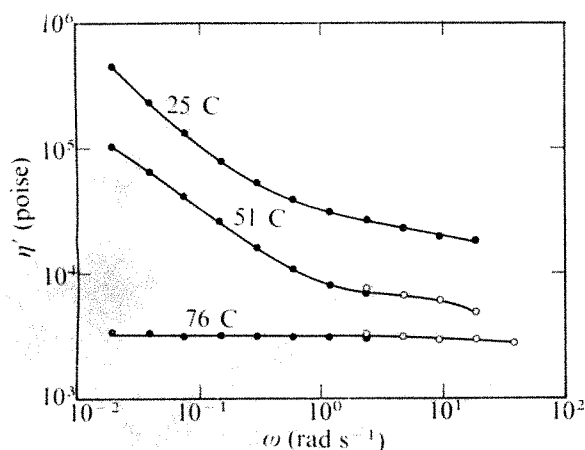


Fig. 1 Dynamic viscosity  $\eta'$  for a styrene-butadiene-styrene block copolymer, with 30% dipentene at temperatures above and below the separation temperature,  $T_s$ . ●, From a chart recorder; ○, from an oscilloscope.

this system,  $T_s$  is between 51 and 76 °C. A light transmittance experiment with the same system using equipment previously described<sup>3</sup> showed a transition in the range 63 to 77 °C, in very good agreement with rheological measurements. Depression of  $T_s$  by solvent, as we observed, seems to explain why 'concentrated' solutions (say, 30% polymer) may exhibit no anomalous behaviour at 35 °C (ref. 3).

The rheological evidence tends to support the interpretation and existence of  $T_s$ , a concept not universally accepted. Rheogoniometry seems to measure  $T_s$  as accurately as previous methods, and identification of  $T_s$  values is essential for the design of fabrication processes. Meanwhile, extension of the Leary-Williams model to plasticised block copolymers is in progress<sup>4</sup>.

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## Drag reduction in laminar flow

WHEN certain long-chain polymers are added to water, the drag exerted by the solution on fixed boundaries is reduced<sup>1</sup>. Although this applies to both internal and external flows, evidence indicates that the effect occurs only in the turbulent regime, at least for steady flow. The work reported here deals with the forced oscillation of a liquid column contained within a semicircular manometer tube, and shows that the addition of a polymer produces a reduction in drag when the flow is laminar.

The apparatus consisted of PVC tubing (3/16 inch bore) arranged in a semicircle (12 inch radius) on a vertical wooden board. The forcing pressure, which is applied to one side of the manometer, is provided by a servo controlled motor which operates rubber bellows through a cam mechanism. The speed of the motor, and thus the forcing frequency, was measured by timing a known number of revolutions of the cam.

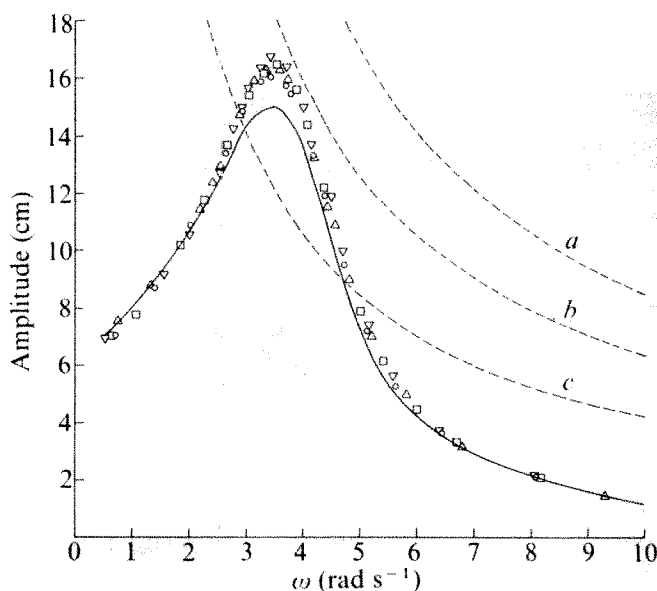
The results are in the form of a resonance test, where the frequency of the forcing pressure is varied and the amplitude of the oscillation of the manometer liquid is measured. Several tests were performed so that the effect of changing the mixture strength could be observed. For each test a new length of tubing was used and the amount of solution in the tube was adjusted so as to preserve a common length of liquid column. Figure 7 shows the results for water and various solutions of Polyox WSR 301 dissolved in water. For the range of solutions tested the polymer produces various degrees of drag reduction, with the weakest solution (12.5 p.p.m.) producing the most effect.

If it is assumed that the displacement of the liquid column is harmonic, then the velocity, and thus the instantaneous Reynolds number, is also harmonic; however, a peak Reynolds number,  $\hat{Re}$ , can be calculated for any given amplitude and frequency, based on the maximum velocity of the liquid column in any one cycle. Thus, if  $x$  is the half-amplitude at a frequency  $\omega$ , then

$$\hat{Re} = 2\omega x r / \nu$$

where  $r$  is the internal radius of the tube and  $\nu$  the kinematic viscosity of the liquid. Thus, lines of constant  $\hat{Re}$  appear as hyperbola in the  $x$ - $\omega$  plane, as shown in Fig. 1. The results are well within the critical  $\hat{Re}$  boundary ( $\sim 2,000$ ) associated with steady flow.

Fig. 1 Amplitude of oscillation as a function of forcing frequency,  $\omega$ , and mixture concentration. —, water; ○, 100 p.p.m.; □, 50 p.p.m.; △, 25 p.p.m.; ▽, 12.5 p.p.m.; a,  $\hat{Re} = 2,000$ ; b,  $\hat{Re} = 1,500$ ; c,  $\hat{Re} = 1,000$ .





The critical Reynolds number for oscillatory flow is frequency dependent<sup>2</sup>, and the parameter which determines the variation from the steady flow case is the Valensi number,  $N_V$ , where  $N_V = \omega r^2/\nu$ .

According to Park and Baird<sup>3</sup> the critical Reynolds number increases from 2,000 when  $N_V > 30$ . In the apparatus described this happens when  $\omega > 7$  so that, for the frequency range in which the drag reduction occurs, the critical Reynolds number should still be about 2,000. It would seem that anomalous results are obtained when comparing the reduction of pipe wall drag for steady and unsteady laminar flows.

We draw attention to this because of its possible application. It is a small part of a more fundamental study under the direction of Dr W. D. McComb<sup>4</sup>, to whom grateful acknowledgement is made.

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## Knowledge of magnetism in pre-Columbian Mesoamerica

THAT the pre-Columbian peoples of Mesoamerica were familiar with the property of magnetism has been suggested by numerous researchers, among them Coe and Fuson<sup>1</sup>. Indeed, a flattened oblong piece of haematite discovered by Coe during the excavation of the Olmec site of San Lorenzo in southern Veracruz state in 1973, has been thoroughly examined by Carlson<sup>2</sup>, who suggests that it probably was manufactured for use as a compass. Fuson has argued that the varied alignments of architectural complexes in many Mayan ceremonial centres may be explained by their having been oriented to compass directions which changed through time, and further, that the Mayas' knowledge of mercury would have permitted them to use vessels filled with this liquid, as well as the water-filled calabashes, to float their 'needles' or lodestones. In January 1975 during field studies at Izapa, in the Pacific coastal plain of south-eastern

Fig. 1 Carvings of snake and turtle heads at Izapa, located 30 m SE of the main pyramid.



Chiapas state, I discovered strong circumstantial evidence that the people of this Late Formative ceremonial centre not only knew about magnetism but possibly even associated it with the homing instinct of the sea-turtle.

While examining astronomical alignments of various of the structures at Izapa, I took a bearing along the axis established by two large sculptures located ~30 m south east of the main pyramid (Fig. 1). In common with the river cobbles that make up the facing of the pyramids and platforms of the site, the sculptures consist of dark brown basalt. Apart from the fact that they are larger and have been specially carved, they are in no way distinguished from the other exposed rock at the site. The smaller of the two sculptures measures 89 cm in length and is unmistakably a representation of a snake head. Some 114 cm behind the snake head, that is, further SE, stands an upright stone stela, roughly squared off but having no discernible

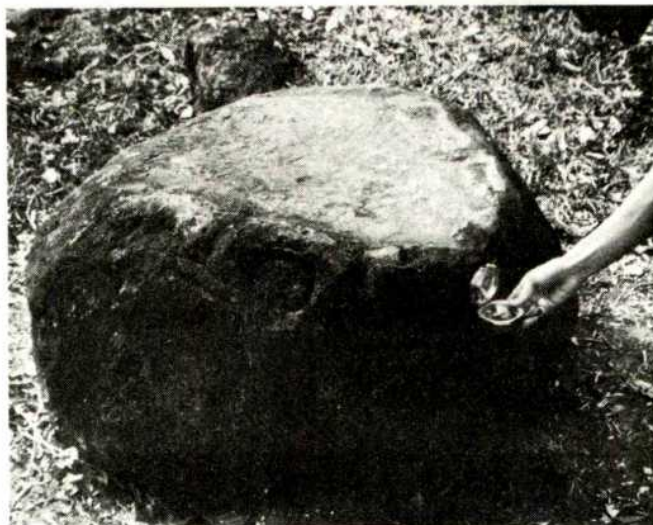


Fig. 2 Close-up of turtle head, with compass needle pointing toward snout.

carvings on any of its faces. A further 256 cm SE is a second stone sculpture, this one measuring 114 cm in length and 122 cm across at its widest point. This stone unquestionably depicts the head of a turtle. When a Brunton compass was brought near the turtle head a sharp deflection of the needle was observed, of more than 60°. No matter where the compass was moved along the perimeter of the sculpture, the needle continuously pointed to the snout of the turtle (Fig. 2). Discovery of this magnetic field prompted the testing of all other exposed rock at the site for magnetic properties, but no others were detected. This would suggest that the Izapans knew about magnetism in that they had reserved a basaltic boulder rich in iron for their carving of the turtle-head, and had executed it so carefully that the magnetic lines of force all came to a focus in the snout of the turtle.

The magnetic turtle-head is not the only representation of this creature found at Izapa. Overlooking the western end of the ceremonial ball court is a large altar carved from a single piece of basalt which is also unmistakably a turtle. A few metres to the south of this altar, adjacent to the wall of the main pyramid, is another sculpture, which has the appearance of an upturned turtle shell, again carved from a single basalt boulder. The latter would obviously have become filled with water during the rainy season, and may well have provided the frictionless surface needed for a shaving or needle of lodestone, floating on a small piece of wood, a leaf, or a straw, to serve as a compass. Clearly the Izapans, a sea-faring people, were impressed by

the navigational ability of turtle<sup>3</sup>, which are common in this area. It may be interesting to note that the theory that turtles navigate by magnetism has not yet been discounted<sup>3</sup>.

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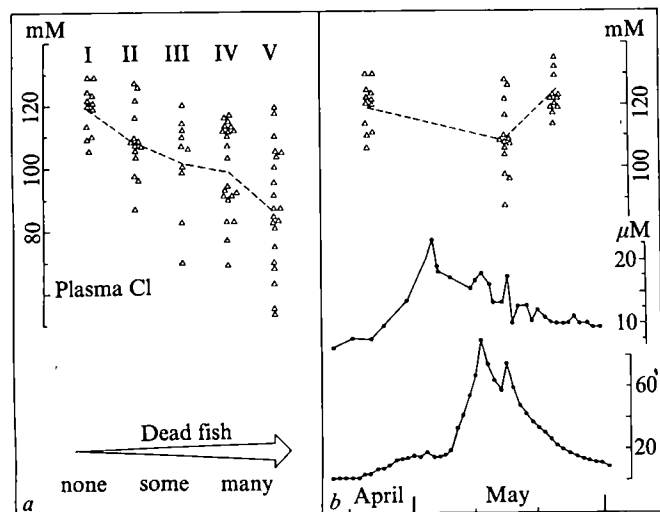
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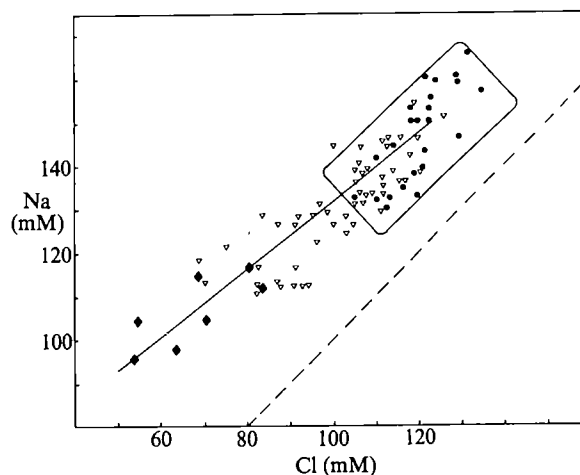
## Fish kill at low pH in a Norwegian river

THE decline in freshwater fish populations in parts of southern Norway is associated with increasing acidity in rivers and lakes<sup>1</sup>. The salmon has been eliminated from many rivers, and hundreds of lakes have lost their trout populations. The chief cause of increased acidity is acid precipitation which is the product of the emission, oxidation and long-distance transport of air pollutants, particularly sulphur dioxide<sup>2,3</sup>. Similar observations of acid rain and the disappearance of freshwater fish populations have been made in the United States, Canada and Sweden<sup>4-6</sup>.

Few cases of massive fish kill due to low pH have been documented in natural environments. A massive fish kill in the Tovdal River in southern Norway in the spring 1975 provided an opportunity to investigate physiological changes in the affected population. Formerly a major salmon river, the Tovdal River is now devoid of salmon, *Salmo salar* L., but still has a population of brown trout,



**Fig. 1** *a*, Chloride content in the blood plasma from *Salmo trutta* collected from different localities along the Tovdal river. The field stations are ranged in order of increasing fish mortality: station V had many dead fish, station I had none. The fish are a fairly representative sample of the population at each locality. Their weight varied between 17 and 250 g. Blood samples were taken by heart puncture in heparinised syringes within 30 min after capture, and the plasma was separated immediately by centrifugation. Chloride was determined coulometrically on 20- $\mu$ l samples. The analyses were performed in a mobile field laboratory. *b*, Water flow in the upper part of the river (station I) before, during and after the major snow melting, April 16 to May 30 ( $\text{m}^3 \text{s}^{-1}$ , lower curve). Acidity of the water was measured as pH and recalculated as  $\mu\text{M H}^+$  (centre curve). Plasma chloride ( $\Delta$ ) from the trout population at station I taken on April 23, May 15 ( $0^\circ \text{C}$ ) and May 23 ( $6^\circ \text{C}$ ).



**Fig. 2** Plasma sodium plotted against chloride from field localities with different degrees of fish mortality. The frame at the upper right corner indicates animals from station I, with no dead fish. Aberrant behaviour includes: lack of avoidance reaction, spiral swimming, and loss of equilibrium at the moment of capture. Plasma cations were determined by atomic absorption spectroscopy at 1/200 sample dilution. The linear regression line drawn has been calculated:  $\text{Na} = 0.746\text{Cl} + 55.35$ ,  $r_{xy} = 0.89$ . — — —, Equimolar concentration for sodium and chloride. Temperature  $0-6^\circ \text{C}$ .  $\bullet$ , No dead fish;  $\Delta$ , many or some dead; diamond, aberrant behaviour.

*Salmo trutta* L. The catchment area of the river is characterised by highly resistant bedrock, mainly granites and gneisses with thin and poorly developed soils. The valley is sparsely populated and has no industry. When the fish kill was noticed, the river was partly covered with ice and the bottom was littered with thousands of dead trout over a stretch of at least 30 km. The fish kill was apparently associated with an influx of pollutants during early phases of snow melting<sup>7</sup>.

Live trout were captured in shallow water by electro-fishing, and blood samples were drawn immediately. Blood plasma was analysed for Na, K, Mg, Ca and Cl. The fish population in the upper reaches of the river was found to be unaffected, and we were thus able to compare this population with samples of surviving trout from the area of severe fish kill. Figure 1 shows that most of the surviving population at the location with the highest incidence of dead fish (station V) had lower plasma chloride contents than those from the unstressed locality (station I). Fish from localities with fewer dead fish show intermediate values (station II, III, and IV). In Fig. 2 the plasma chloride has been plotted against sodium for all the field data. As might be expected, the decrease in plasma chloride is accompanied by a reduction in sodium. The calculated regression shows that for each chloride ion lost there is a decrease in sodium of 0.75 ions.

At the upper reaches of the river (station I) the snow started to melt on April 21, and continued at a moderate rate until May 6 (Fig. 1b). The increased acidity shown in Fig. 1b during the early phase of melting was accompanied by a similar increase in sulphate and other components associated with acidic precipitation. During this early phase of melting, pollutants accumulated in the snow pack are leached out<sup>7</sup> and may reach the river unchanged because of frost in the ground. Blood samples taken on May 15, contained significantly less plasma sodium and chloride than samples taken before and after the snow melting. The pH had dropped from 5.2 to a minimum of 4.65 ( $23 \mu\text{M}$ ) in the early phase of melting, but values as low as pH 4.0 ( $100 \mu\text{M}$ ) was registered in some of the small tributaries to the main river.



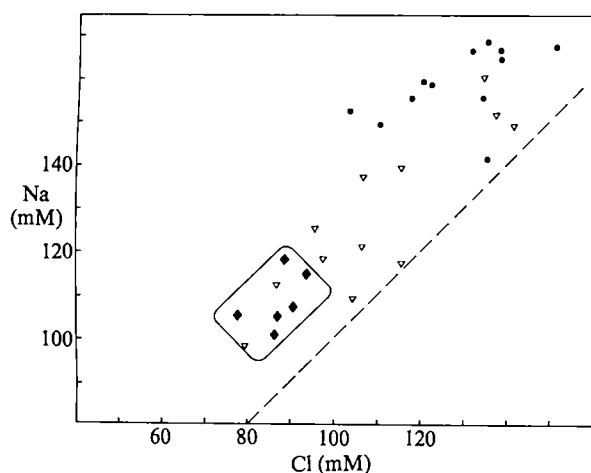


Fig. 3 Plasma sodium plotted against chloride from tank experiments conducted in July 1974 at 18 °C. Trout kept at pH 6.0 and trout exposed to pH 4.0 for 20–100 h. The pH was regulated by metered addition of sulphuric acid. The broken line indicates equimolar concentration for sodium and chloride. "Dying" animals have lost their ability for integrated bodily movements. The frame gives the range in concentration for "dying" animals. ●, pH 6.0; △, pH 4.0; diamond, pH 4.0 "dying".

Fish from a locality with high incidence of dead fish (station V) were transferred to tanks with running water of the same water quality, but neutralised to pH 6.0 by metered addition of dilute potassium hydroxide. After 2 weeks at this pH, the previously stressed fish had regained their normal sodium chloride levels. The change in plasma ions induced by varying only the pH indicates that high acidity is the dominating stress agent for fish in the river.

Brown trout from the wild stock population of the Tovdal River has been used in tank experiments at different times of the year. Fish kept in tanks with water acidified to pH 4.0 lose their ability to regulate plasma sodium and chloride concentrations (Fig. 3). The lowest sodium chloride levels were always found in animals so heavily stressed that the ability for integrated bodily movements had been lost.

Freshwater fish take up ions actively through the gill epithelium. Sodium is exchanged for hydrogen ions and chloride for bicarbonate<sup>8</sup>. Increased hydrogen ion activity in the surrounding medium will impede the active uptake of sodium<sup>9,10</sup>.

This study has shown that low plasma sodium chloride is associated with massive fish kills and with exposure to low pH in tank experiments and in the field. The plasma content of potassium, calcium and magnesium is not affected. It seems probable therefore that impairment of the active transport mechanism for sodium and/or chloride ions through the gill epithelium is the primary cause of fish death in acid waters. Severe ionic imbalance is known to affect fundamental physiological processes such as nervous conduction and enzymatic reactions. The pH below which fish populations are affected depends on several factors. There are differences between species—salmon is more sensitive than trout. Newly hatched fry are more sensitive than adult fish and lack of recruitment is common in acid lakes<sup>1,6</sup>.

It should be stressed that the lakes and rivers that have lost their fish populations contain small amounts of dissolved salts. Typical values for specific conductance is 10–30  $\mu\text{mho cm}^{-1}$ , total hardness is 0.9–6.0  $\text{mg l}^{-1}$  as  $\text{CaCO}_3$  and Na is 0.5–2  $\text{mg l}^{-1}$ . Not only are such waters poorly buffered but field surveys suggest that the fish population is affected at a higher pH in lakes with extremely low ion content. In the spring, pollutants released in the first phases of snow melt produce a pH shock in the rivers and surface

layers of lakes. The subsequent meltwater lowers the total ion content and thus reinforces the pH stress. The fish kill in the Tovdal River indicates that this is the most critical season for the fish populations.

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## Chemical treatment of soil alleviates effects of soil compaction on pea seedling growth

HIGH mechanical resistance of soils, related to naturally occurring hard pans, or to compaction by machinery and untimely cultivation<sup>1–3</sup>, often reduces crop yields through poor development of root systems<sup>1,4–6</sup>. In the laboratory, progressive increases in mechanical resistance of the growth medium cause reduced root elongation<sup>7–9</sup>, stunting and thickening of root systems<sup>3,10</sup> and reduced shoot growth<sup>11</sup>. Loosening of subsoil in the field can reduce compaction, increase aeration and waterholding capacity of the soil and improve the distribution of available water<sup>1</sup>. Roots can then penetrate to greater depths and crop yields often increase<sup>1,12,13</sup>. White light also affects root growth, inhibiting axial extension in some species<sup>14–16</sup>. This effect can be counteracted in cross roots by 3,5-diiodo-4-hydroxybenzoic acid (DIHB)<sup>17–19</sup>. The fact that compacted soil and white light can both induce stunting and thickening of root systems suggested to us that similar growth control mechanisms might be involved. We have therefore investigated whether DIHB can improve root development in compacted soils; we have shown that this is the case.

The roots and shoots of pea seedlings grown in compacted control soil (moistened with water) in growth room conditions were found to be much shorter and had smaller dry weights than those of seedlings grown in loose control soil (Tables 1–3). Furthermore, the growth-inhibited roots were thickened and bent whereas the roots of seedlings grown in loose control soil were slender and straight. These responses of roots to adverse soil conditions are typical of those observed in various crop plants<sup>3,8,10,11</sup>.

Addition of  $10^{-4}$  M or  $10^{-6}$  M solutions of DIHB to soil followed by compaction, reduced the level of root growth inhibition, the overall lengths of unstraightened roots being 19% and 31% greater, respectively, than those of corresponding roots in the compacted control soil. When the roots were straightened the chemical was found to have induced increases of 19% and 24% (Table 1). Similar DIHB treatments applied to the loose soil had little detectable effect on root elongation at either  $10^{-4}$  M or  $10^{-6}$  M, root length in both cases being the same as in the loose control soil (Table 2). Clearly, therefore, DIHB enhanced root penetration in compacted soil and was ineffective in loose soil.

Straightening and measuring the roots of pea seedlings grown in  $10^{-4}$  M DIHB compacted soil or the compacted

**Table 1** Effect of DIHB solution or distilled water incorporated in a compacted silt loam soil on the length of pea seedling roots

DIHB concentration (M)	Length (mm)	Shoot % Increase over control	Length (mm)		Root % Increase over control	
			Unstraightened	Straightened	Unstraightened	Straightened
Control	14.12±0.78	—	16.90±0.91	23.97±1.07	—	—
10 <sup>-6</sup>	15.37±1.02	8.85	22.12±1.34	29.63±1.36	30.89	23.61
10 <sup>-4</sup>	15.04±0.91	6.51	20.06±0.95	28.56±1.12	18.70	19.14

Data are the means and standard errors from six samples each containing at least eight seedlings. Silt loam soil of the Wood Series was taken from the plough layer (0–22.5 cm), sieved (4 mm mesh) and air dried. Gravimetric moisture determinations were carried out so that the final soil moisture content could be adjusted to 20% (pF 3.6) by weight using distilled water or aqueous DIHB solution. Treated or control soil was placed in rigid plastic isodiametric pots (7.5 cm high, 10.2 cm inner diameter, Osma) in four layers of equal weight and each layer was compressed evenly using an air-operated piston. Each pot constructed in this way was filled to a depth of 6.5 cm. Applied pressure at the soil surface was 3.16 kg cm<sup>-2</sup> for compacted soil and 0.15 kg cm<sup>-2</sup> for 'loose' soil (read from a standard curve showing the relationship between soil bulk density and moisture content over a range of applied pressures for the Wood Series soil<sup>20</sup>), which gave dry bulk densities of 1.5 and 1.1 g cm<sup>-3</sup> respectively. Seeds of *Pisum sativum* L. cv. Meteor were soaked in flowing tap water for 4 h before germination between sheets of moistened Whatman No. 1 filter paper at 25±1 °C in darkness. After 22–24 h, seedlings with radicles 2 mm long were planted in the pots containing control or DIHB-treated soil. Radicles were embedded firmly in small holes made in the soil surface and the pots were filled to capacity with the appropriate soil, which was gently firmed by the piston at a pressure of 0.15 kg cm<sup>-2</sup>. Polythene sheet was used to cover the pots to prevent evaporation of water from the soil. After further incubation for 72 h at 20±2 °C, seedlings were removed from the soil and washed in tap water. Root length of seedlings grown in compacted soil was assessed as (a) the length of a straight line extending from the base of the root to the root tip, thereby not accounting for any deviations due to bending and (b) the entire length of the root after straightening. These two values, which are referred to as unstraightened and straightened lengths respectively, gave a guide to the amount of bending of the root induced by the soil conditions. Roots of seedlings grown in loose soil were free from such bending so only the length of the straight root was recorded.

control soil revealed a 42% increase in length; for roots grown in the 10<sup>-6</sup> M DIHB compacted soil treatment the increase was 34% (Table 1). Since a highly bent root would show a large increase in overall length on straightening and vice versa, it follows that the roots grown in the 10<sup>-6</sup> M DIHB compacted soil were less bent than those from either the 10<sup>-4</sup> M DIHB or the control compacted soils. Thus the concentration of DIHB which was most effective in enhancing root elongation through the compacted soil also reduced root bending in response to high mechanical resistance of the soil.

A trend was detected which indicated that small increases in shoot length occurred when seedlings were grown in compacted soil treated with 10<sup>-4</sup> M and 10<sup>-6</sup> M DIHB, but the chemical had little effect on shoot length in the loose soil treatments (Tables 1 and 2).

Seedling dry weights were not affected by inclusion of DIHB solution in the loose soil treatments, but increases in the weight of both shoots and roots were detected in the DIHB compacted soil treatments (Table 3). The greatest dry weight increase was found in seedlings subjected to the 10<sup>-6</sup> M DIHB compacted soil treatment, which, as we have already seen, was also the most effective treatment for enhancement of root and shoot elongation.

We have demonstrated, therefore, that incorporation of DIHB into compacted soil can produce substantial increases in overall root length of pea seedlings and small increases in shoot length and seedling dry weight. DIHB can thus reduce root growth inhibition induced by both compacted soil conditions and exposure to white light. This supports our initial idea that the two responses might have similar control mechanisms. Investigations in our laboratories have shown that DIHB may counteract effects of light on root

elongation by reducing ethylene-induced growth inhibition<sup>19,21</sup>. Ethylene can also cause swelling and bending of roots<sup>22,23</sup> and these distortions are similar to those observed in roots of seedlings growing in compacted soils<sup>3,10</sup>. Anaerobic conditions arising as a result of conditions prevailing in some compacted soils can stimulate the production of ethylene by soil microflora<sup>20,24,25</sup> and mechanical resistance can also induce endogenous production of ethylene by roots and shoots<sup>23,26</sup>. Thus, it is possible that DIHB acts by reducing the production and/or the inhibiting effects of ethylene derived from the soil or the plant tissue or both. Further research is required to assess the relative

**Table 3** Dry weights of root and shoot tissues of pea seedlings treated with DIHB

DIHB concentration (M)	Dry weight per seedling (mg)			
	Compacted		Loose	
	Shoot	Root	Shoot	Root
Control	8.41±0.72	5.54±0.52	9.77±0.45	7.66±0.31
10 <sup>-6</sup>	10.99±1.17	6.50±0.72	9.96±0.35	7.92±0.18
10 <sup>-4</sup>	9.55±0.86	6.03±0.53	10.14±0.15	7.87±0.08

Pea seedlings were grown for 72 h at 20 °C in 'loose' or compacted silt loam soil, treated with DIHB solution or distilled water (control) to give a 20% soil moisture content. Data for each treatment are the means ± s.e. from six samples, each consisting of at least eight seedlings.

importance of mechanical resistance, ethylene and anaerobic conditions in limiting seedling growth, and also to determine whether DIHB relieves growth inhibition due to one or more of these factors. The moisture content of the soil used in the present studies, however, was well below that at which pea seedling growth is limited through lack of aeration<sup>9,28</sup> and in view of the short growth periods involved it seems unlikely that anaerobic conditions would be established. Whatever the reason, the important aspect of our findings is that seedling growth can be improved by treating compacted soil with low concentrations of specific chemicals, an effect which is not apparent in non-compacted soil.

Intensive investigations are now in progress to determine the conditions necessary for optimum plant response to DIHB and the precise mode of action of the chemical and its analogues 3,5-Dichloro- and 3,5-dibromo-4-hydroxybenzoic acids, for example, have much the same effect on pea seedling growth in compacted soil as DIHB and the results of these studies will be published in full elsewhere.

**Table 2** Effect of DIHB solution or distilled water incorporated in a 'loose' silt loam soil on the elongation of pea roots and shoots

DIHB concentration (M)	Shoot		Root	
	Length (mm)	% Change over control	Length (mm)	% Change over control
Control	21.47±0.56	—	65.98±1.82	—
10 <sup>-6</sup>	21.37±0.46	-0.47	64.55±1.59	-2.17
10 <sup>-4</sup>	21.24±0.49	-1.07	67.04±1.44	+1.60

The density of the soil was 1.1 g cm<sup>-3</sup>, and its moisture level was 20%. Seedlings were grown for 72 h at 20 °C and the data for each treatment are the means ± s.e. from six samples each consisting of at least eight seedlings.

Clearly, these findings of enhanced seedling performance in adverse soil conditions could open exciting new possibilities in practical agriculture by helping to combat the effects of soil compaction on plant growth using chemical rather than mechanical means.

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## Substance stimulating the differentiation of spores of the blue-green alga *Cylindrospermum licheniforme*

THE study of intercellular inductions in higher plants and animals is hindered by the complexity of the multicellular tissues involved. Sporulation of filamentous blue-green algae is a relatively simple process to study because these algae contain, in linear array, only three types of cells. There are vegetative cells, and heterocysts and spores which form by differentiation of vegetative cells. Wolk<sup>1,2</sup> showed that heterocysts of the blue-green alga *Anabaena cylindrica* Lemm. induce adjacent vegetative cells to differentiate into spores. We report here that a substance produced by the related alga, *Cylindrospermum licheniforme*, greatly stimulates sporulation of that same alga.

Stock cultures *C. licheniforme* Kutz. were grown axenically<sup>3</sup> in an eightfold dilution of the medium of Allen and Arnon<sup>4</sup> on a rotatory shaker at  $27 \pm 1^\circ \text{C}$ , illuminated with cool-white fluorescent light at an intensity of 2,200 lx. These cultures, subcultured 1% v/v weekly to maintain a density below  $2.5 \mu\text{g}$  chlorophyll per ml, contained no spores.

Phosphate deficiency stimulates sporulation of *Cylindrospermum*. Cultures of *C. licheniforme* sporulated in phosphate-free standard sporulation medium (SSM)<sup>1</sup> containing 5.7 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid (TES), pH 7.5, as buffer in place of DL-alanyl-glycine. Conditioned SSM was the centrifugal supernatant fluid from 50 ml 10–15-d-old cultures, and from 250 ml

15–20-d-old cultures. SSM and conditioned SSM were inoculated (2%, v/v) from 7-d-old stock cultures, and were cultured in the same conditions of temperature and light as were the stock cultures. In some experiments, conditioned SSM was supplemented with all the constituents of SSM, or was lyophilised and then made up to its original volume by addition of SSM or water.

In *C. licheniforme*, heterocysts normally develop at the ends of filaments, and spores differentiate first next to the heterocysts. The extent of sporulation was quantified as the percentage of heterocysts with an adjacent spore or spores. (Heterocysts can be caused to form at an intercalary position<sup>5</sup>, in which case spores form on both sides of these heterocysts, but intercalary heterocysts were not observed as part of our experiments.) Cells were considered to be heterocysts only if they contained the polar granule<sup>6</sup> characteristic of heterocysts, and were considered to be spores only if they were at least twice as long as a normal vegetative cell, and showed no sign of septation. Growth was measured as an increase in the chlorophyll content of a culture, as determined with methanolic extracts<sup>7</sup>.

Filaments grown in SSM show no acceleration of sporulation until the fifth day of culture, whereas the acceleration of sporulation of filaments grown in conditioned SSM starts essentially immediately (Fig. 1). The final percentage sporulation is approximately the same in the two cultures. Growth and sporulation of *C. licheniforme* in SSM therefore modifies (conditions) that medium in a way that makes it stimulatory to spore formation on reinoculation. No significant difference in growth accompanies the difference in sporulation. Therefore, stimulation of sporulation cannot be due to an effect on growth. Conditioning also had no significant effect on the pH of SSM.

Supplementation of conditioned SSM with all the constituents of SSM does not affect the sporulation-stimulation which results from conditioning (Table 1). Supplemented, conditioned SSM contains all of the components of SSM in concentrations equal to or greater than their concentration in SSM. The stimulation of sporulation which is effected by conditioning of SSM is not, therefore, the result of a depletion of the medium. Rather, the filaments produce a substance which is released into the medium and which stimulates the differentiation of spores in newly inoculated filaments. We conjecture that sporulation of the original inoculum results from an increasing concentration of this same substance.

A 1:19 dilution of conditioned SSM with SSM retains 50% of its sporulation-accelerating activity. Lyophilised samples retain activity after storage for at least 1 month at room temperature, reconstitution with water or SSM, and sterilisation by autoclaving.

Blue-green algae release a large amount of polypeptidic and other organic material into their medium<sup>8–12</sup>. The substance produced by *C. licheniforme* may function normally as an extracellular agent, an intrafilamentous agent, or both. As an extracellular agent, it might become more concentrated during stagnation or desiccation of a natural body of water, and thereby signal the need for

Table 1 Effect of supplementing conditioned medium with components of fresh sporulation medium

Medium	% Heterocysts with spores	
	A	B
Standard sporulation medium (SSM)	$6.4 \pm 2.7^*$	$5.8 \pm 2.6^*$
Conditioned SSM	$66.0 \pm 6.1$	$64.8 \pm 6.6$
Conditioned, supplemented SSM	$68.0 \pm 13.8$	$67.2 \pm 12.8$

In experiments A, constituents of SSM were added to liquid, conditioned SSM. In experiments B, liquid SSM was added to lyophilised, conditioned SSM.

\* Mean  $\pm$  s.e., from five experiments, each of which was assayed after 3 d of culture.

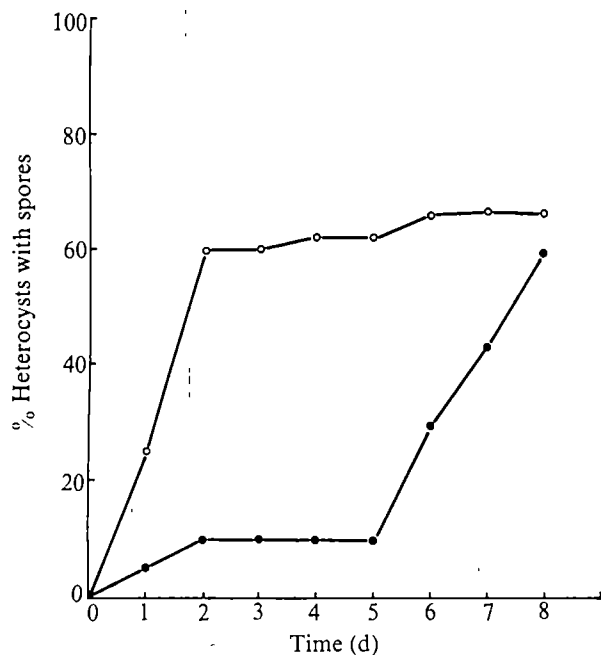


Fig. 1 Time course of sporulation of *C. licheniforme* in SSM (●) and in SSM conditioned by previous growth and sporulation of the alga (○).

*C. licheniforme* to sporulate so as to survive an impending period of dryness. Within filaments, the substance may be involved in the possible induction, by heterocysts, of the sporulation of adjacent vegetative cells<sup>13</sup>. Thus, if heterocysts induce adjacent vegetative cells to sporulate by producing this substance, and if some of it were to leak out, the exogenous supply could accelerate sporulation in a second inoculum by supplementing the supply, within vegetative cells, which comes from heterocysts. Alternatively, the juxtaposition of the first-formed spores to heterocysts may mean that the heterocysts control the entry of, activation of, or response to, the exogenous factor.

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## Inference of the time of origin of some *Drosophila* species

MUCH attention has recently been paid to the interpretation of protein evolution through amino acid substitution. These events are under genetic control and seem, in certain well documented cases, to occur at a constant rate per site per

year for many species<sup>1,2</sup>. At the population level, arguments have been adduced that the observed allozymic variation may also have similar clock-like properties<sup>3</sup>. A further area of present interest is the existence of certain pairs of species which show only very slight genetic difference as measured by allozymes<sup>4–6</sup>. These cases may represent pairs of newly formed species. Thus, genetic similarity may serve as direct measure of the recency of the cladistic event which separated the two compared lines of descent. This note extends the 'protein clock' idea to a series of species endemic to a set of successively younger island volcanoes, for which ages are well documented.

The ages of each of the twelve volcanoes comprising the five largest Hawaiian Islands (Kauai, Oahu, Molokai, Maui and Hawaii; see inset, upper left, Fig. 1) are accurately known from potassium-argon and magnetic declination data<sup>7</sup>. According to a well-supported theory, each volcano has been formed in sequence from north-west to south-east. They have emerged above the ocean in the order listed above, as the Pacific crustal plate has drifted north-westwards over a fixed 'hot spot' in the Earth's mantle<sup>8</sup>. The ages of the oldest rocks of each volcano<sup>7</sup> can be read along the horizontal axis in Fig. 1.

Eight very closely related but morphologically distinct (that is, non-sibling) species of the *Drosophila planitibia* subgroup are found on these islands<sup>9,10</sup>. They show very narrow geographical distributions, being restricted to the volcanoes listed under each species name on Fig. 1. The island of Hawaii shows no lava flows older than 700,000 yr (ref. 11), so the two sympatric species endemic to the island of Hawaii must be relatively newly formed. These species, *D. heteroneura* and *silvestris*, are, moreover, virtually indistinguishable electrophoretically<sup>5</sup>. *D. heteroneura*, probably the newest species in the series, has been selected as a standard for pairwise comparisons with each of the other seven species. Genetic similarities based on allozymes (Rogers<sup>12</sup> coefficient *S*) have been used in these comparisons.

Along the ordinate in Fig. 1 is given the genetic distance, *D*, obtained in pairwise comparisons between *heteroneura* and each of the other seven species. Following Nei's<sup>13</sup> suggestion, *D* has been calculated as the negative natural logarithm of the similarity coefficient. Differences calculated from the data<sup>5</sup> have been plotted against time, measured by the age of the oldest known emergent rocks of each volcano or island. The time of each cladistic event (splitting) is indicated along a diagonal line, *A*, in Fig. 1. This line assumes allozymic identity at separation and then an accumulation of genetic difference directly proportional to the elapsed time since the cladistic event. Diagonal *A* assumes that genetic difference accumulates at a rate of 1% in 20,000 yr. These are large, slow-breeding flies of high, cool rain forests; there may be no more than two generations a year.

These assumptions fit the age of the islands, the endemic position of the species and the chromosomal phylogenies reasonably well. For example, the cladistic event separating *D. substenoptera* of Oahu from the line leading from *picticornis* of Kauai towards *heteroneura* would have occurred when Kauai and West Oahu (Waianae) were the only existing emergent volcanoes. *D. neopicta*, a pivotal ancestor common to the more advanced species in the chromosomal phylogeny<sup>10</sup>, would have been, as expected, the first of the series to colonise Molokai. At first glance, *hemipeza* seems, from the electrophoretic data alone, not to be old enough to have evolved ancestrally on Oahu. This is, however, in accord with earlier theories based on chromosomes<sup>9,10</sup>. Thus *hemipeza* has been considered a 'back-migrant', having colonised Oahu relatively recently from a Molokai ancestor.

Diagonal dashed lines *B* and *C* (Fig. 1) assumes a slower and faster rate of change respectively. *B* shows an accumu-



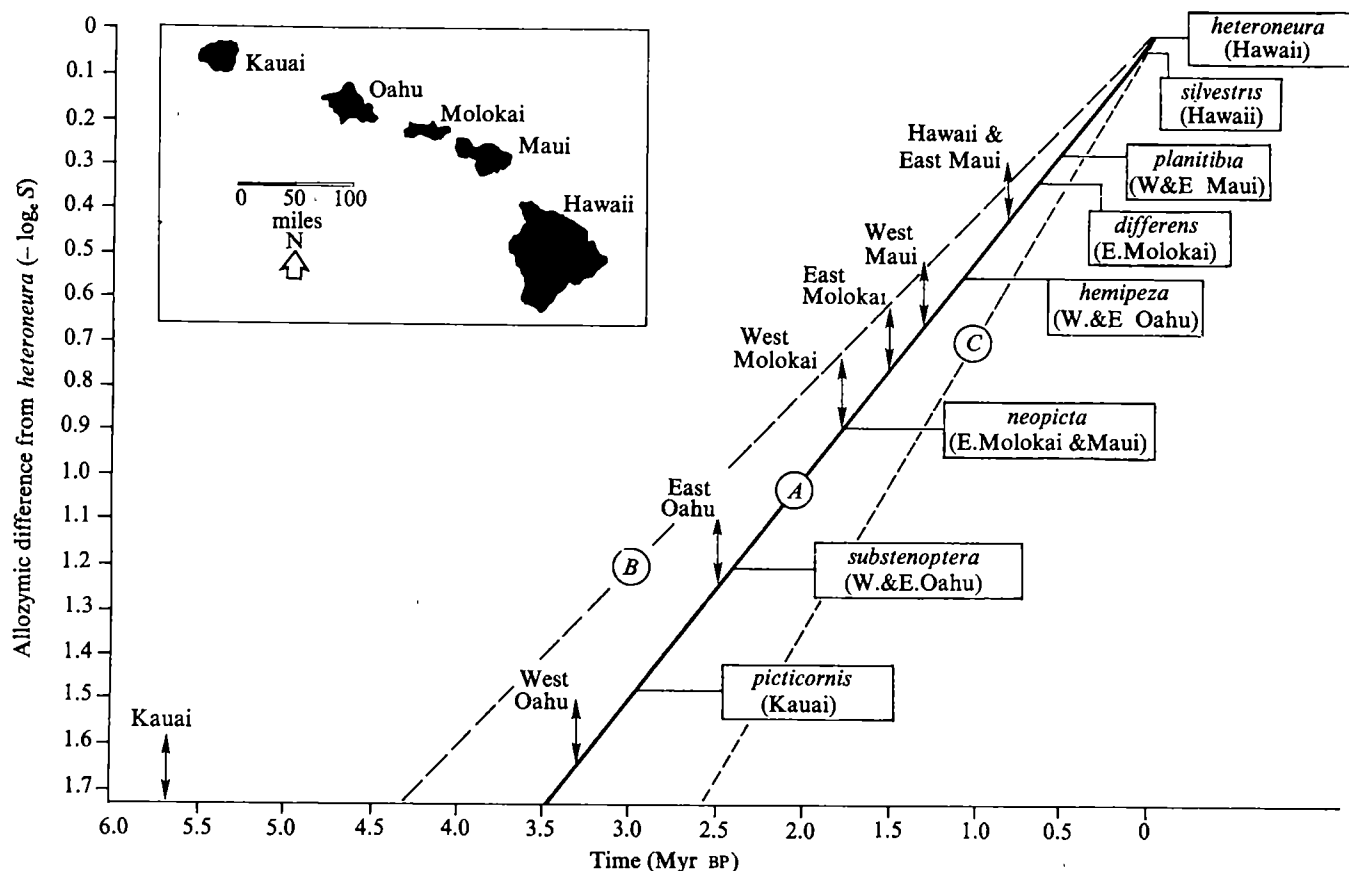


Fig. 1 Allozymic difference and time of species origin in eight species of Hawaiian *Drosophila* (*planitibia* subgroup).

lation genetic difference at the rate of 1% in 25,000 yr and C a rate of 1% in 15,000 yr. Both of these assumptions produce a less satisfactory fit of the data on differences to the geological, geographical and chromosomal data. The rate which fits best, suggests that a level of  $D=1.0$  may be attained after two million years of separation. This is about 15 times faster than the rate suggested by another well-documented case<sup>8</sup>.

Accordingly, the following cautious suggestion may be made. Accumulation of electrophoretically detected protein differences over geologic time may possibly serve as a useful clock mechanism, permitting details of the past evolutionary history of a species to be inferred from the biochemical state of the living forms.

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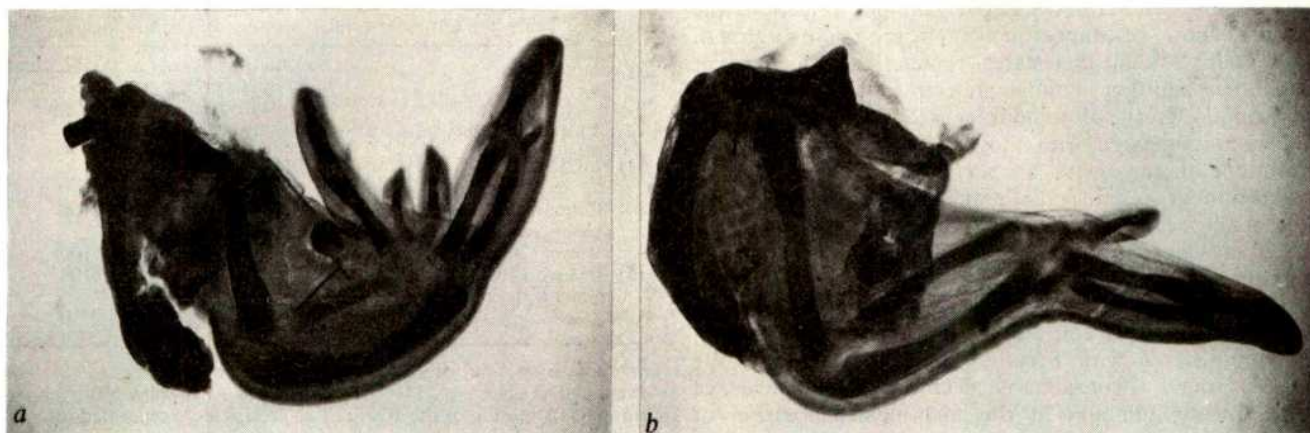
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## Positional signalling by mouse limb polarising region in the chick wing bud

THE development of the chick limb may be viewed in terms of positional information; that is, the spatial pattern of cellular differentiation results from the cells interpreting their position in a three-dimensional coordinate system<sup>1</sup>. We have presented evidence that positional information along the antero-posterior axis is specified by a signal from the zone of polarising activity (ZPA), which is at the posterior margin of the wing bud<sup>2,3</sup>. Grafts of ZPA to anterior regions of an early bud result in mirror image duplication between grafted ZPA and host ZPA which is particularly evident in the pattern of the digits. The three digits of the chick wing<sup>4</sup>, digits 2, 3, and 4 appear to be specified by their distance from the nearest ZPA, digit 4 being closest, then digit 3 and then digit 2. The ZPA from the leg can also specify additional wing digits when grafted into the wing which suggests that the positional information along the antero-posterior axis in leg and wing is similar, but that interpretation differs. We therefore wanted to know whether a ZPA was present in other classes of vertebrates, and if so whether the signal from it was also similar. We investigated this question by grafting tissue from embryonic mouse limbs into developing chick wing buds.

Arms and legs of mouse embryos aged 10 d (the day of the vaginal plug was taken as day 0) were used to provide tissue to graft into the chick wing. These limbs were chosen as, morphologically<sup>5</sup>, they resemble stages of the chick wing which have been shown to possess a ZPA. As far as we can judge from the work of Jurand<sup>6</sup>, the time taken to lay down the pattern of the forelimb is approximately the same for both chicks and mice. The arms and legs were cut off



**Fig. 1** Dorsal views of whole mounts of operated wings stained to show cartilage pattern. *a*, Wing resulting from a graft of a mouse arm ZPA showing a pattern of digits 32234. Note there is a small nodule of cartilage at the anterior margin of the wing, but not an identifiable digit 4. *b*, Wing resulting from a graft of a piece of tissue from the anterior margin of a mouse arm showing a normal pattern of digits, 234. The little hooks are the pins used to keep the grafts in place.

the mouse embryos, and regions from posterior margins of the limbs  $\sim 300\mu\text{m}$  from the tip were tested for ZPA activity. This region was taken because it would correspond to the ZPA of the chick limb bud. These small pieces of mouse limb tissue were pinned into holes cut into the anterior margins of embryonic chick wings (stage 20–22) opposite somite 16 in contact with the apical ectodermal ridge. (In this position with an implanted chick ZPA the pattern of digits in the resultant wing has been shown<sup>3</sup> to be 432234 or 43234.) In a few cases the graft was placed opposite somite 17. The host chick embryos were then incubated for a further 5 d at 37 °C; then they were killed and the pattern of the digits of the operated wing was examined. From 20 grafts of these regions from mouse limbs, we obtained 13 wings with extra digits; 5 wings with normal digits; and 2 wings with extra pieces of cartilage which could not be identified as particular digits. Of the 13 grafts which produced wings with extra digits, 6 wings had the digit pattern 2234 and the remaining 7 wings 32234 or 3234 or 334 (see Fig. 1*a*). Regions of both arms and legs of mouse embryos were used in these experiments, and there was no detectable difference in the ability of these to specify additional digits in the wing. As a control, we took equivalent pieces of tissue from the anterior margin of mouse legs and arms and grafted these into chick wings in the same way. All 7 of these wings were normal, with no extra digits (see Fig. 1*b*). In five additional grafts, more proximal pieces of tissue from the posterior margins of the mouse limbs were assayed and these operated wings were normal.

These results show that the embryonic mouse limb possesses a ZPA and its position is similar to that in the embryonic chick wing. Moreover, this mouse ZPA can specify additional digits in the chick wing. We would emphasise that the response to a grafted ZPA has thus far not been obtained by grafting any other tissue, even though a wide variety have been tested. Such test tissues include: tissues from other regions of the chick limb, other embryonic chick tissues such as liver and brain, and rat endocrine organs.

The pattern of digits specified by the mouse ZPA must be compared with that specified by a chick ZPA. There was no way of predicting the effect of the mouse ZPA in the chick limb, since we know neither the relative level of the signal, nor the threshold for specifying the various digits of the mouse. Whereas with a chick ZPA opposite somite 16 one almost always obtains a well formed digit 4 adjacent to the graft<sup>3</sup>, with the mouse ZPA this never occurred. There is reason to believe that the signal from the ZPA is graded, and falls off with distance<sup>3</sup>. Whenever we have effectively reduced the signal, by placing the chick

ZPA further away from competent tissue, or by interfering with its propagation by Nucleopore filters, digit 4 is not formed, but digits 3 and 2 may develop. Thus the inability of the mouse ZPA to specify digit 4 may be caused either by its specifying an intrinsically lower positional value, or by the transmission of the signal being impaired.

Slack (personal communication) has recently shown that there is a region similar to the ZPA in early amphibian embryos, and one may thus reasonably infer that a ZPA is present in all vertebrates with limbs. Our results suggest that the signal from it may be the same in all vertebrates, the differences along the antero-posterior axis being due to differences in interpretation. In this connection it is of interest that mouse dermis can interact with chick epidermis to produce feather buds from the epidermis<sup>7,8</sup>, and Waddington<sup>9</sup> showed that Hensen's node from a rabbit gave rise to a secondary neural tube when implanted into a chick host of the same developmental stage.

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## Suppression of the incidence of death with spontaneous tumours in DBA/2 mice after *Corynebacterium parvum*-mediated rejection of syngeneic tumours

KILLED *Corynebacterium parvum* has been effective when administered into tumours or when included as a subcutaneous inoculum containing tumour cells (the subcutaneous site, unlike the intradermal site<sup>1</sup>, facilitates early



tumour cell dissemination)<sup>2-4</sup>. After tumour rejection, the augmented host resistance prevented progressive growth of potentially lethal metastases present at the time of therapy<sup>5</sup>. In addition, animals rejecting tumours formed from primary grafts of tumour cells admixed with killed *C. parvum* (compared with those rejecting tumours after intratumour therapy with *C. parvum*) exhibited an augmented tumour-specific protection against inoculation of large challenge cell doses<sup>6</sup>. Whitmire and Huebner<sup>7</sup> reported a significant decrease in incidence of 3-methylcholanthrene-induced malignancies in C57BL/Cum and BALB/c C<sub>v</sub> mice after preimmunisation with formalin-killed wild-type virus and Rauscher leukaemia virus vaccines administered with Freund's complete adjuvant. We have now found a considerable decrease in the incidence of spontaneous tumours in the high-incidence strain of DBA/2 mice after rejection of tumours formed from subcutaneous transplants of syngeneic mammary adenocarcinoma cells admixed with killed *C. parvum*.

DBA/2 female mice (4-6 weeks old, Jackson Laboratory) and the well differentiated syngeneic T1699 mammary adenocarcinoma (Jackson Laboratory) were used. Seven hundred and fifty previously unsensitised mice were separated into five equal groups and received subcutaneous injections as described in Table 1. We included 60 unsensitised mice which received 10<sup>6</sup> living tumour cells alone. The experiment was repeated 3 months after the start of the initial studies. In addition (Table 2) three groups of 50 unsensitised mice received subcutaneous injections as for groups 1, 4 and 5. They were evaluated for tumour-specific resistance.

Of the five groups (Table 1), mice receiving subcutaneous living tumour cells and killed *C. parvum* (group 5) developed tumours at the injection site. Tumours grew for 11 d (mean) followed by their rejection in all animals as previously described<sup>2</sup>. Only four of the 150 mice in group 5 developed spontaneous tumours (mammary adenocarcinomas) at 10-13 months of age. They exhibited minimal increase in tumour size (from the day of origin) for approximately 60 d (mean) followed by accelerated tumour growth and death with growing tumours and metastases (survival range from onset of tumour was 77-96 d). Mice given irradiated tumour cells mixed with *C. parvum* (group 4) exhibited no tumour growth at first but 49 developed spontaneous tumours at 9-13 months (with growth patterns

**Table 1** Incidence of death from spontaneous tumours in DBA/2 mice

Group (150 mice per group)	Deaths from spontaneous tumours	
	Experiment 1	Experiment 2
(1) 0.9% NaCl	105	117
(2) Killed <i>C. parvum</i> (200 µg) alone	96	107
(3) Irradiated T1699 cells (10 <sup>6</sup> ) alone	101	112
(4) Irradiated T1699 mixed with killed <i>C. parvum</i> (200 µg)	49	56
(5) 10 <sup>6</sup> Living T1699 cells mixed with killed <i>C. parvum</i> (200 µg)	4	9
10 <sup>6</sup> Living T1699* cells (60 mice)	60	60

\*All animals died with growing tumours and metastases after injection of tumour cells.

Effect of *C. parvum*-mediated rejection of T1699 mammary adenocarcinoma cells on death with spontaneously growing tumours in DBA/2 mice. Groups of DBA/2 mice (150 animals each) received subcutaneous injections (0.2 ml) of one of the following (1) 0.9% NaCl; (2) killed *C. parvum* (200 µg) and 0.9% NaCl; (3) 10<sup>6</sup> irradiated T1699 tumour cells (10,000 r.); (4) 10<sup>6</sup> irradiated T1699 cells with killed *C. parvum* (200 µg), and (5) 10<sup>6</sup> living T1699 cells with killed *C. parvum* (200 µg). A further 60 mice received 10<sup>6</sup> living T1699 cells alone. The animals were inspected weekly for 30 months or until death with or without growing tumours. All animals receiving living tumour cells mixed with killed *C. parvum* exhibited tumour growth (at injection site) for 11 d, followed by tumour rejection. Mice receiving tumour cells alone died with growing tumours and metastases.

**Table 2** Tumour-specific resistance in DBA/2 mice

Initial injection	Second injection (5 mice per group) Live 10 <sup>7</sup> tumour cells	Survival after second sub- cutaneous injection (%)
(1) NaCl (0.9%)	T1699 cells	0
	CAD <sub>2</sub> cells	0
(4) 10 <sup>6</sup> Irradiated T1699 tumour cells mixed with killed <i>C. parvum</i> (200 µg)	T1699 cells	100
	CAD <sub>2</sub> cells	100
(5) 10 <sup>6</sup> Live T1699 tumour cells mixed with killed <i>C. parvum</i>	T1699 cells	100
	CAD <sub>2</sub> cells	0

Tumour-specific protection in DBA/2 mice after *C. parvum*-mediated rejection of T1699 tumours. At 20 months, 10 surviving animals from each of the above three groups were separated into two subgroups of five mice each. They then received subcutaneous injections (0.2 ml) of either 10<sup>7</sup> T1699 or 10<sup>7</sup> CAD<sub>2</sub> cells.

similar to those in group 5) followed by death with growing tumours and metastases (range of survival after onset of tumour was 70-102 d). The remaining three groups had higher incidences of spontaneous tumours. Of these, 105 treated with 0.9% NaCl (group 1) developed tumours at 8-15 months and died with growing tumours and metastases (range of survival from onset of tumour was 40-60 d). The groups treated with killed *C. parvum* (group 2) or irradiated tumour cells (group 3) exhibited similar incidences of spontaneous tumours and survival after onset of tumour (range: *C. parvum*, 32-35 d; irradiated T1699 cells, 44-67 d). Findings were similar in experiment 2. Histological sections from random autopsies revealed that all tumours were mammary adenocarcinomas with various degrees of differentiation. The 60 mice receiving 10<sup>6</sup> living T1699 cells alone (in experiments 1 and 2) all died with growing tumours and metastases between 28 and 48 d after inoculation of tumour cells.

At 20 months of age, 10 surviving animals (Table 2) from each of the three groups (50 per group) given treatments approximate to groups 1, 4 and 5 were separated into two subgroups of five. All mice of a subgroup received subcutaneous injections of either 10<sup>7</sup> live T1699 or 10<sup>7</sup> CAD<sub>2</sub> cells. The CAD<sub>2</sub> mammary adenocarcinoma syngeneic to DBA/2 mice is antigenically different from the T1699 tumour. The characteristics of this tumour which originated spontaneously in a DBA/2 female have been described before<sup>8</sup>. The results (Table 2) show that the animals that did not develop spontaneous tumours (after rejection of tumours formed from grafts of T1699 cells admixed with *C. parvum*: group 5) were protected and did not develop tumours after reinjection of 10<sup>7</sup> live T1699 cells. Findings were similar when the surviving animals from group 4 received a second injection of T1699 cells. But, the subgroups of mice in groups 4 and 5 (Table 2) that received a second injection of 10<sup>7</sup> living CAD<sub>2</sub> cells were not protected and died with growing tumours and metastases as did the mice in group 1 which received either CAD<sub>2</sub> or T1699 cells. All surviving mice (experiments 1 and 2) were dead at the end of 30 months with no tumours.

Thus DBA/2 mice exhibited a 3-6% incidence of death from spontaneous tumours after rejection of tumours formed from grafts of syngeneic mammary adenocarcinoma cells mixed with killed *C. parvum*. Furthermore, mice given irradiated syngeneic tumour cells mixed with killed *C. parvum* exhibited a 50% reduction in that incidence of death with spontaneous tumours compared with the control groups. In addition, mice given either irradiated or living tumour cells mixed with killed *C. parvum* exhibited tumour-specific protection which was evident for 20 months suggesting that the conferred host resistance was of long duration.

These findings were probably associated with the immunostimulant adjuvant properties of *C. parvum*

administered in association with living tumour cells. Thereafter, the augmented response conferred as a result of rejection of a large mass of tumour may have been directed by both virus-specific and tumour-specific transplantation antigens associated with these tumour cells and continued to protect the host from death with spontaneously growing tumours, or perhaps prevented progressive growth of newly formed tumour cells *in vitro*. Evidence for this comes from the demonstration that pretreatment of experimental animals with these viral or tumour antigens protected the recipients against tumour growth from syngeneic cell grafts<sup>9-13</sup>. Considerably less protection was afforded the mice treated with irradiated tumour cells mixed with killed *C. parvum*, which may have reflected a weakened response towards a decreased tumour cell antigen mass, but may correlate with studies showing that optimal usefulness was observed in experimental animals when living tumour cells were used in specific active immunotherapy<sup>17,18</sup>.

The immunopotentiating properties of *C. parvum* in association with tumour cells also conferred augmented host resistance<sup>19</sup>. This vaccine when administered to experimental animals activated both the reticuloendothelial system and phagocytosis for long periods. In addition stem cells were stimulated to differentiate into macrophages in these animals; T and B cells were stimulated to proliferate and recruitment of lymphocytes was stimulated<sup>20-22</sup>. *C. parvum* also stimulated the primitive surveillance mechanisms that have been found to be effective in tumour rejection<sup>19,23,24</sup>.

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## Immunosuppression by micromolecular fibrinogen degradation products in cancer

PATIENTS with advanced cancer and lymphoid malignancies often show impaired cellular and humoral immune responses, partly caused by serum factors inhibiting lymphocyte reactivity<sup>1-3</sup>, which, although not fully defined, are considered to be low molecular weight (poly)peptides.

Another frequent finding in malignant diseases is the appearance of circulating fibrin(ogen) degradation products (FDPs)<sup>4,5</sup>. Besides the commonly investigated breakdown products X, Y, D and E, plasmin liberates several small peptides from human fibrinogen, designated "micromolecular FDPs"<sup>6</sup>. These are dialysable, thermostable, have molecular weights between 15,000 and 500 (ref. 6) and have distinct physiological properties<sup>7-10</sup>. Since both the degree of impairment of lymphocyte function and the frequency of FDP formation are related to the extent of neoplastic spread<sup>11</sup>, we investigated the influence of micromolecular FDPs on cellular and humoral immune responses. A striking immunosuppressive activity was found, possibly responsible for impaired immunoresponsiveness in cancer patients. Terminal FDPs were obtained by prolonged digestion of human fibrinogen (Forschungsfibrinogen Kabi) with human plasmin (Forschungsplasmin Kabi) in aqueous solution, pH 7.4, and dialysed against distilled water at 4 °C for 24 h. The dialysate was concentrated by evaporation in a vacuum, reconstituted in physiological saline, sterilised by filtration and added to lymphocyte cultures using a microculture system<sup>11</sup>. As Fig. 1 shows, micromolecular FDPs caused a dose-dependent suppression of PHA-induced lymphocyte transformation, which was 98% at 1.1 mg ml<sup>-1</sup>. These concentrations did not affect cell viability as measured by Trypan blue exclusion. Plasmin dialysates alone, prepared in identical conditions, inhibited neither of the systems tested: separation of fibrinogen on Sephadex G-200 showed no evidence of pre-existing contaminants (Fig. 4).

To characterise the active components, micromolecular FDPs were separated on Sephadex G-25. All preparations revealed four peaks (1, 2, 3 and 4), the first appearing with the void volume (Fig. 2). Peaks were reduced to the initial volume of the sample and tested in the lymphocyte culture. A marked inhibitory activity was always found in peak 3; peaks 1 and 4 were slightly suppressive.

Additional experiments were designed to test the effect of micromolecular FDP on sheep red blood cell (SRBC)-specific primary immune responses in Swiss mice. Animals

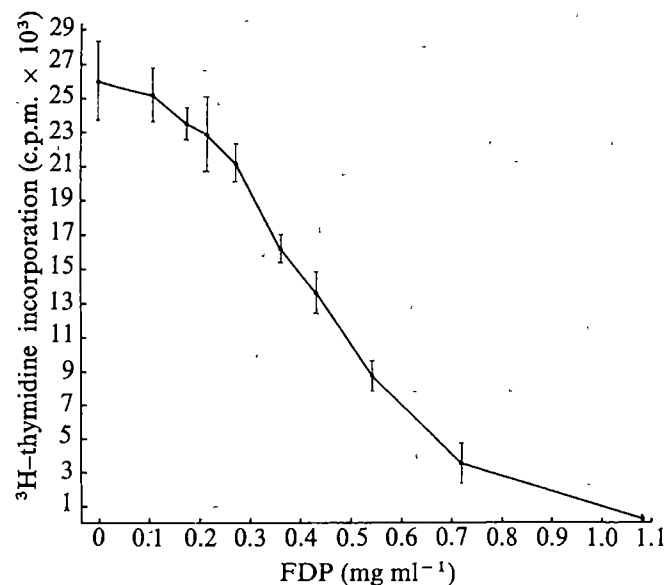


Fig. 1 Dose-dependent suppression of PHA-induced <sup>3</sup>H-thymidine incorporation of human peripheral lymphocytes by increasing amounts of micromolecular FDPs. Protein concentrations were assayed according to Lowry *et al.*<sup>19</sup>. After purification on Ficoll-Hypaque 200,000 cells were cultured in 0.1 ml of Eagle's MEM with 10% pool human AB serum using 0.1 ml of PHA-P (Difco) as a stimulant (25 µg per ml culture fluid). Samples of 20 µl of FDP dilutions in saline were added; controls included saline instead of FDPs and MEM instead of PHA. Cultures were collected after 66 h.



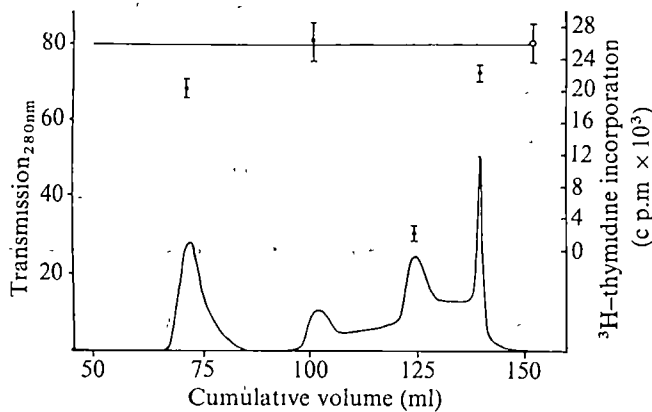


Fig. 2 Inhibitory activity of micromolecular FDPs separated by Sephadex G-25 gel filtration on PHA-induced lymphocyte transformation. Chromatography was carried out using column K 16 (Pharmacia, void volume 65 ml) and distilled water as eluant. Peaks were concentrated by evaporation in a vacuum, reconstituted with saline in the original sample volume, and tested as described in Fig. 1. ○, c.p.m. of controls with saline; ●, c.p.m. of cell cultures with FDPs added (mean  $\pm$  s.d.).

were immunised intraperitoneally with  $4 \times 10^8$  SRBCs and simultaneously injected intravenously with various dilutions of FDP. The splenic content of SRBC-specific plaque-forming cells (anti-SRBC PFC) was determined according to Jerne and Nordin<sup>12</sup>. The mean number of anti-SRBC PFC per  $10^6$  spleen cells in primed controls was 1,820 (Fig. 3). Unprimed controls either treated with FDP ( $n=3$ ) or untreated ( $n=6$ ) produced only few "background-plaques" (6–12 PFC per  $10^6$  cells). Treatment with 100  $\mu$ g of FDPs did not alter significantly the SRBC-specific immune response, whereas administration of 130–160  $\mu$ g markedly inhibited PFC production (mean value of 998 PFC per  $10^6$  cells).

To test the effect of different timing on specific PFC production, this dose was administered at different times before and after primary immunisation. The most striking immunosuppression (about 80%) occurred in mice treated with 130–160  $\mu$ g of FDPs 24 h after priming, whereas PFC responses in animals treated with 100  $\mu$ g of FDPs were not significantly different from the control value (Fig. 3).

These findings clearly demonstrate a time-dependent and dose-dependent immunosuppressive action of micromolecular FDP. In this experimental model FDPs act predominantly in the early phase of clonal proliferation, that is 24 h after antigenic challenge. By this time, DNA synthesis will be activated, but significant antibody production is still lacking.

Since we suspected a relationship between immunosuppressive activity and circulating FDPs in the blood of cancer patients, we studied 19 donors with malignant diseases and three patients with hepatic cirrhosis to look

for a correlation between both findings. Because direct measurements of micromolecular FDP are not yet available, we determined the higher molecular weight FDPs in plasma by means of reptilase time, thrombin coagulase time and the ethanol gel test, and in serum using the Laurell technique after collecting blood with Aprotinin.

Serum samples were fractionated by Sephadex G-200 chromatography, and the fourth peak containing the peptide material<sup>1</sup> (Fig. 4) was further chromatographed, on Sephadex G-25 as described for FDP. Chromatography of some sera revealed a first peak (A) corresponding to peak 1 of FDP; in all sera except one, two further peaks appeared, one of which (B) corresponded to peak 3, the other (C)

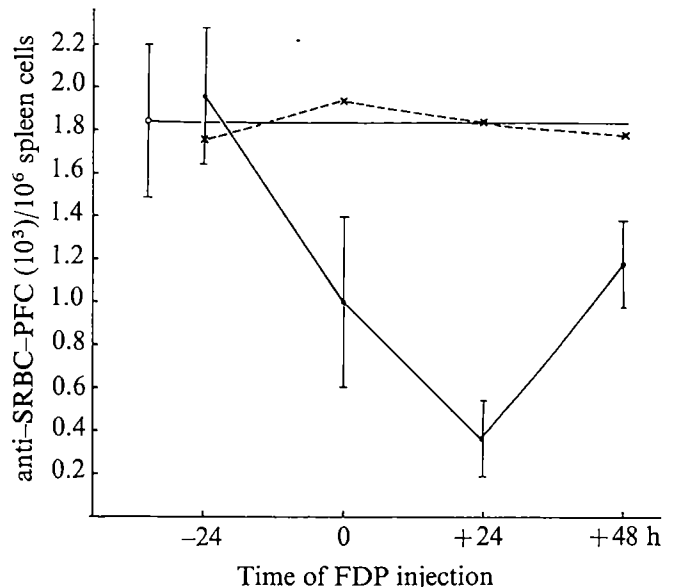


Fig. 3 Dose-dependent and time-dependent suppression of splenic production of anti-SRBC PFC by micromolecular FDPs in Swiss mice. FDP dilutions were administered intravenously in a single injection at the intervals indicated with respect to intraperitoneal primary immunisation with  $4 \times 10^8$  SRBC. Data represent day-4 PFC responses per  $10^6$  spleen cells (vertical bars indicate s.d.) calculated for groups of six mice. Primed animals receiving no further treatment served as controls ( $n=22$ ). ○, Controls; ●, 130–160  $\mu$ g FDP; ×, 100  $\mu$ g FDP.

to peak 4 of micromolecular FDPs. In the peak 2 region of FDPs no material appeared. Peaks and sera were again added to lymphocyte cultures, and a significant immunosuppressive activity was defined as at least 40% inhibition of PHA-induced  $^3$ H-thymidine incorporation.

Representative experiments are shown in Table 1. The sera and/or Sephadex G-25 peaks of seven patients without signs of circulating FDPs (cases 1–7) revealed no immunosuppressive activity, whereas those of eleven patients with demonstrable FDP (cases 8–18) inhibited lymphocyte transformation markedly. In only four cases investigated so far

Table 1 Occurrence of FDP and of inhibitory plasmatic activity (representative cases)

Case	Reptilase time (s)	Thrombin coag time (s)	Ethanol gel test	FDP Laurell (mg%)	% Inhibition by serum or peaks of rechromatography			
					Serum	peak A	peak B	peak C
2	14.3	18.1	Neg.	0	15	—	—	28.5
5	11.5	16.8	Neg.	0	24.9	—	0	0
6	17.9	18.9	Neg.	0	32.6	—	32.4	32.6
12	27.3	26.0	Pos.	10.0	45.4	—	71.3	16.0
16	32.3	30.9	Pos.	19.0	68.5	—	99.6	68.4
18	21.4	19.8	N.D.	25.0	N.D.	—	98.6	11.9

Diagnosis of all patients investigated: Chronic lymphocytic leukaemia (1,4), anaplastic Ca unknown origin (2), chronic uraemia with Ca rectum (3), Ca kidney (5,14), Hodgkin's disease (6,10,15,20), metastatic Ca mamma (7), liver cirrhosis (8,9,21), metastatic adeno-Ca unknown origin (11), malignoma suspected (12), metastatic solid Ca unknown origin (13), Ca pancreas (16,17), Ca bronchus (18,19), adeno-Ca stomach (22). —, No peak eluted in this position N.D., not done. For normals reptilase time and thrombin coagulase time were 22–24 s; ethanol gel test was negative and FDP Laurell was not measurable.

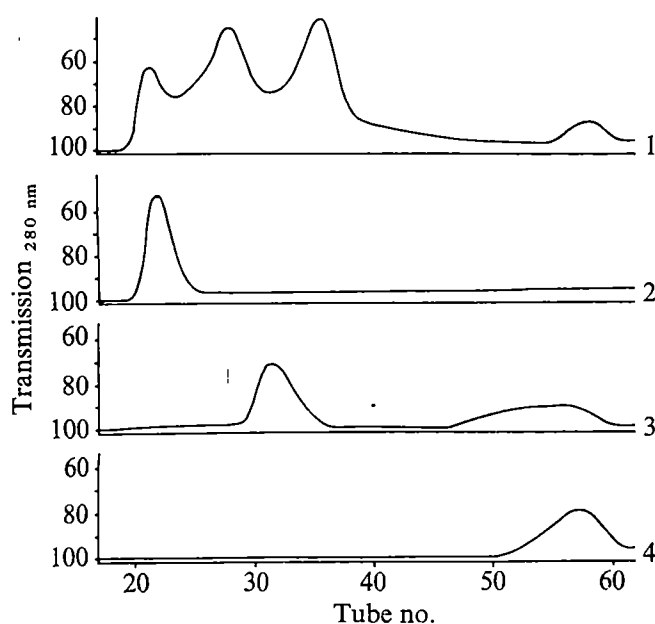


Fig. 4 Elution patterns (Sephadex G-200) of a patient's serum (curve 1), fibrinogen Kabi before digestion (curve 2), plasmin-digested fibrinogen (curve 3), and the micromolecular FDP separated by dialysis (curve 4). Column K 16 (Pharmacia), 0.1 M Tris-buffer, pH 7.4 as eluant. The micromolecular immunosuppressive molecules appear during plasmin digestion of fibrinogen, the dialysable moiety of which is eluted in the identical position as serum peak 4.

(19–22) was no direct correlation between inhibitory serum factors and circulating FDP evident. The inhibitory activity was most frequently localised in peak B, corresponding to the maximal inhibitory region of FDP eluates. Certain cases—especially with evidence of high FDP values in plasma—showed additional or predominant inhibition in peak C.

Our preliminary results suggest that micromolecular FDP, which are known to inhibit thrombocyte aggregation<sup>7,9,10</sup> and to influence the tonus of smooth muscles<sup>8</sup>, also have immunosuppressive activity. According to separation characteristics on Sephadex G-25, their molecular weight ranges below 5,000. Whether these peptides are identical with other serum inhibitors, not yet characterised definitively, needs further investigation. Clearly, however, in most conditions for which the presence of immunosuppressive serum factors has been reported, fibrinogen degradation products do indeed occur—in carcinomas<sup>2,4</sup>, Hodgkin's diseases<sup>3,13</sup>, renal diseases<sup>14,15</sup>, pregnancy<sup>5,16</sup> and transplant rejection<sup>17,18</sup>. Furthermore, it seems remarkable that in two of our cases with a non-neoplastic disease (hepatic cirrhosis) FDP-containing sera markedly inhibited lymphocyte transformation.

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## Correlation between specific cytotoxicity and expression of idiotypic receptors of allograft-infiltrating cells

REJECTION of an allograft is normally accompanied by infiltration of the graft by host cells. The origin, type and receptor specificity of these cells as well as their precise functions are largely unknown, primarily because of the extreme difficulties of quantitative recovery of the infiltrating cells. A promising experimental technique<sup>1</sup> has recently been developed: a spongy matrix tissue is infiltrated by strain A fibroblasts, transplanted into B animals and removed at various times thereafter. Mere physical compression of the 'sponge' releases virtually all of the infiltrating B cells whereas virtually none of the A fibroblasts will be released. The released B-strain cells are functionally intact.

It has been found possible to visualise and enumerate lymphoid cells with specific immune reactivity toward major histocompatibility locus-determined antigens<sup>2,3</sup>. Such visualisation is achieved by the use of specific anti-idiotypic antibodies selectively reacting with B and T lymphocytes carrying idiotypic receptors signifying reactivity against a certain alloantigen. We here describe the cytolytic capacity and idiotype expression of cells invading an *in vivo* sponge 'allograft'.

Fibroblasts were allowed to invade sponge matrix allografts by implantation into the peritoneal cavities of DA, Lewis or BN rats. These strains differ at the major histocompatibility locus<sup>4</sup>. Five days later the sponges were removed and transplanted subcutaneously into the neck of Lewis or DA rats. These rats were killed seven or eight days later (at peak killer activity in the graft (P. J. Roberts and P.H., unpublished)) and the sponges, as well as the draining (axillary-inguinal) and non-draining (popliteal) lymph nodes and the spleens were removed. Cell suspensions were prepared as described previously.

Table 1 Expression of anti-DA idiotypic receptors on graft-infiltrating cells, on draining and non-draining lymph node cells and on spleen cells seven days after grafting of a syngeneic or allogeneic sponge matrix graft

	Graft	Source of cells* Draining lymph nodes	Non-draining lymph nodes	Spleen
Lewis carrying DA graft	56.0	25.1	4.5	9.1
DA carrying Lewis graft	1.3	0.7	0.0	0.1
Lewis carrying Lewis graft	2.0	5.8	5.0	4.9
Lewis carrying BN graft	3.9	7.5	5.4	5.7
Normal Lewis rat	ND	ND	4.5	5.2
Normal DA rat	ND	ND	0.1	0.2

\*Figures refer to the percentages of cells stained by direct immunofluorescence with FITC-conjugated anti-(anti-DA) antiserum<sup>3</sup>. ND, not done.

The percentages of cells displaying idiotypic receptors of Lewis-anti-DA specificity in the various cell-suspensions were analysed using FITC-conjugated anti-Lewis-anti-DA IgG antibodies. As seen in Table 1, low 'background noise' staining was observed in the control DA rats (highest percentage 'positive' cells being 1.3% in the most macrophage-rich population). In contrast, the Lewis cells from normal rats, or from rats carrying BN grafts, were all within the previously found range of percentage<sup>3</sup> (2–7.5% idiotype positive cells). In Lewis cell populations from rats receiving DA sponges, a normal (4.5% anti-DA) idiotypic frequency in the non-draining nodes, a slight increase in the spleen, and highly significant increases in the draining node cells and particularly among the sponge-invading cells were recorded. Thus, a highly selective increase of cells expressing idiotypes signifying anti-DA reactivity were indeed found infiltrating DA grafts transplanted into Lewis rats. Two similar experiments yielded essentially similar results, with a variation in the data of  $\leq 20\%$ .

In a second series of experiments cells invading a sponge allograft (and cells from the various lymphoid organs) were

analysed for idiotype-positive cells, as well as for specific cytolytic activity *in vitro* against relevant target cells. To further define the participating effector cells in this system, an investigation was carried out to determine the percentage of phagocytic or IgG-coated, latex positive (bound on the surface) cells among the idiotype-positive and negative cells. The results of such experiments are shown in Table 2. A clearcut positive correlation between the occurrence of an increased percentage of cells with anti-DA idiotypes and specific anti-DA cytolytic capacity of the same cell suspension was observed (see the results obtained using cells from Lewis rats grafted with DA sponges). When the capacity to bind latex particles on the cell surface and/or phagocytic activity were investigated, striking differences were observed between idiotype-positive cell populations obtained from the various rats and organs.

Lewis-anti-DA idiotype-positive cells obtained from lymph nodes or spleens always contained a majority of latex negative cells irrespective of whether the Lewis rats had, or had not, received any BN or DA sponges. This is to be expected since Lewis-anti-DA idiotype-positive T cells

**Table 2** Percentage anti-DA idiotype carrying cells and cytolytic anti-DA activity seven days after grafting of syngeneic or allogeneic sponges. Distribution of receptors for Fc of Ig and phagocytosis in idiotype-positive and negative cells

Rat	Source of cells Organ	Total anti-DA idiotype positive* (%)	Percentage distribution of latex-positive and phagocytic† cells within the idiotype-positive or negative cells				Cytolytic‡ anti-DA activity
			Expression of idiotype	Latex negative	Latex positive on cell surface	Latex positive phagocytosing	
Lewis carrying DA graft	Graft	42.8	pos.	51.2	7.2	41.6	44.8
			neg.	49.1	3.2	47.8	
	Draining lymph node	21.1	pos.	72.0	13.0	13.0	22.5
			neg.	96.0	2.0	1.0	
	Non-draining lymph node	5.2	pos.	92.3	1.9	5.8	4.8
			neg.	88.8	10.6	0.7	
	Spleen	9.2	pos.	72.8	21.7	5.4	26.0
			neg.	58.3	37.3	4.4	
DA carrying Lewis graft	Graft	1.3	pos.	0.0	7.7	92.3	ND
			neg.	56.1	2.1	41.7	
	Draining lymph node	0.7	pos.	0.0	0.0	100.0	ND
			neg.	80.4	9.4	10.3	
	Non-draining lymph node	0.0	pos.	ND	ND	ND	ND
			neg.	88.4	11.3	0.3	
	Spleen	0.1	pos.	0.0	0.0	100.0	ND
			neg.	64.4	31.3	4.3	
Lewis carrying Lewis graft	Graft	2.0	pos.	60.0	40.0	0.0	-0.9
			neg.	50.7	3.0	46.2	
	Draining lymph node	5.8	pos.	86.2	12.1	1.7	1.2
			neg.	79.2	9.9	10.9	
	Non-draining lymph node	5.0	pos.	86.0	14.0	0.0	ND
			neg.	85.5	14.1	0.4	
	Spleen	4.9	pos.	67.4	18.4	14.3	0.4
			neg.	63.0	33.6	3.4	
Lewis carrying BN graft	Graft	3.9	pos.	43.6	18.0	38.5	5.3
			neg.	49.1	4.5	46.4	
	Draining lymph node	7.5	pos.	80.0	6.7	13.3	3.4
			neg.	81.3	8.6	10.1	
	Non-draining lymph node	5.4	pos.	90.8	7.4	1.8	2.2
			neg.	87.4	11.3	1.3	
	Spleen	5.7	pos.	73.7	17.5	8.8	1.3
			neg.	61.3	33.6	5.1	
Normal Lewis rat	Lymph node	4.5	pos.	91.1	6.7	2.2	ND
			neg.	87.5	10.8	1.7	
	Spleen	5.2	pos.	71.8	19.3	9.6	ND
			neg.	63.5	32.3	4.1	
Normal DA rat	Lymph node	0.1	pos.	0.0	0.0	100.0	ND
			neg.	85.6	13.7	0.7	
	Spleen	0.2	pos.	0.0	0.0	100.0	ND
			neg.	63.8	31.4	4.8	

\*Total number of anti-Id carrying cells in a given sample by direct immunofluorescence with FITC-conjugated anti-(anti-DA) antiserum.

†The cells were first incubated at 37 °C for 30 min with IgG-coated latex particles. After preparation of the cell smears, each cell was investigated for the presence/absence of surface immunofluorescence, for the presence/absence of latex particles attached on to the cell surface ('Fc-receptor' carrying cells) and for latex particles ingested inside the cell (phagocytic cells). All cells phagocytosing latex particles also attached latex particles on their surface.

‡The killer assay was run overnight in V-shaped microtitre plates using a killer-target cell ratio of 100:1 using <sup>51</sup>Cr-labelled PHA blasts as target cells, with the media and killer conditioned essentially as described before<sup>5</sup>.

normally outnumber B cells carrying the same idiotype by a factor of 5–7 (ref. 3). Graft-invasive idiotype-negative cells had, on average, a higher percentage of latex-positive, phagocytic cells than cell suspensions obtained from lymph nodes or spleens. The 'background' noise of false 'positive' anti-DA idiotype cells in the fluorescent antibody technique was caused by latex-positive, phagocytic cells (see, for instance, figures obtained using cells from DA rats receiving Lewis sponge). We interpret this false positive staining to arise from complexed FITC-IgG molecules being bound by way of Fc receptors. In the DA grafts transplanted into Lewis rats, however, a very high percentage of the total population of graft-invasive cells displayed anti-DA idiotypes as well as the capacity to bind latex particles on the cell surface and phagocytic activity (compare with composition of cells extracted from BN sponges). The exact nature of these latter idiotype-positive, latex-binding and phagocytic cells is unknown, but they may very likely be of macrophage origin. If so, the presence of idiotype-positive material on the surface of the DA sponge invading macrophages would still be obscure (whether from specific T cells<sup>6</sup> or from surface binding of complexes made up of Lewis-anti-DA alloantibodies and DA alloantigen). Also, it would remain to be established whether such idiotype-coated macrophages could act like specifically armed macrophages with regard to DA target cells. Fractionation of the various cell types with subsequent analysis for cytotoxic activity are under way to determine these questions.

In conclusion we can state that an allograft is invaded by a complex variety of cells carrying actively produced or passively adsorbed idiotype receptors on the surface with specific reactivity for the target alloantigens. A positive correlation could be found between the presence of increased numbers of such idiotype-positive cells and the specific cytolytic activity against the relevant alloantigen.

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## Cytotoxicity of antibody-coated trypanosomes by normal human lymphoid cells

DESTRUCTION of antibody-coated mammalian target cells by normal human lymphocytes demonstrated by release of <sup>51</sup>Cr-chromate is a phenomenon characterised by its rapidity of action and by the very small amounts of antibody required. It seems unlikely that such a mechanism destroys solely mammalian target cells, and possibly it has a role in immunity to parasitic infections. Butterworth *et al.*<sup>1</sup> demonstrated that normal human blood cells destroy

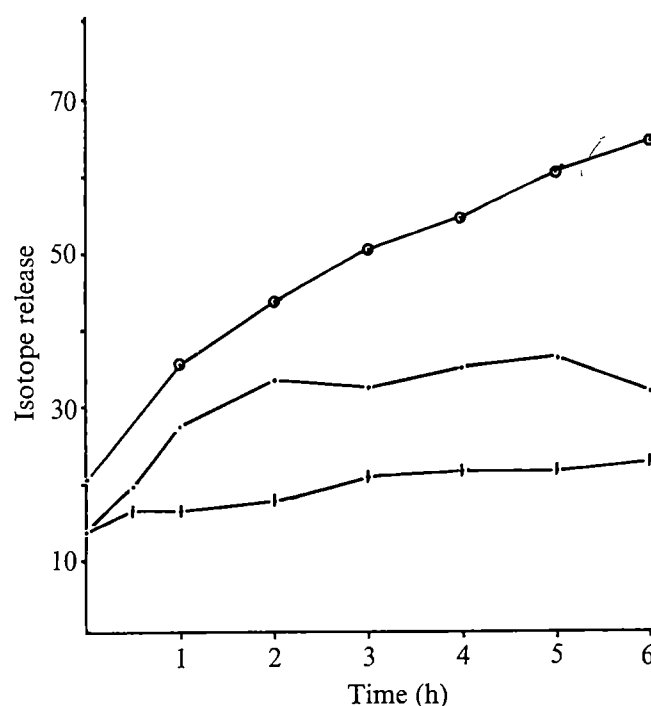


Fig. 1 Time course of isotope release from *T. dionisii* culture forms. Circle and dot, isotope release from parasites incubated in the presence of antibody (1/100 dilution) and normal human lymphocytes; dot, parasites incubated in the presence of normal human lymphocytes alone; vertical line, incubated in medium alone. Statistical summary: analysis of variance—interaction of time with treatment (antibody, lymphocytes or medium) very significant ( $P < 0.05\%$ ;  $F = 5.5$ , d.f. 14 and 72; limiting value for  $F = 3.82$ ). Loge least significant differences for comparison of means up to 0.239, corresponding to a difference of 5.3% at 20% isotope release, 10.7% at 40% and 16% at 60%.

schistosomula coated with antibody from infected patients.

We present here evidence that normal human lymphoid cells in the presence of specific antibody can cause isotope release from culture forms of *Trypanosoma* (*Schizotrypanum*) *dionisii*, a parasite related to *T. cruzi* with an intracellular stage in its vertebrate host (bats).

Protozoa do not take up enough <sup>51</sup>Cr-chromate to make it possible to use this label in cytotoxicity experiments. Technetium (as <sup>99m</sup>Tc-pertechnetate) can be used as a label for target cells in cytotoxicity experiments if non-radioactive sodium chromate is present in the labelling mixture (unpublished results of D.F., B. Tayabali and P. O'Brien); the target cells are presumably labelled with some complex organometallic ion of chromium and technetium. The isotope is released when the cells are killed, is not reutilised and, like <sup>51</sup>Cr-chromate, gradually leaks out of target cells. Repeated freezing and thawing causes the release of about 70 or 80% of the isotope, and treatment of labelled protozoa with detergent Nonidet causes the release of all the isotope. In all respects, therefore, protozoa labelled with technetium in this way behave like mammalian cells labelled with either <sup>51</sup>Cr-chromate or <sup>99m</sup>Tc-pertechnetate.

Culture forms of *T. dionisii* were grown in a liquid medium (L4N (ref. 2) with the concentration of serum and erythrocyte lysate reduced by half). They were washed three times and incubated with about 1 mCi of sterile <sup>99m</sup>Tc-pertechnetate eluted from a generator (Radiochemical Centre). To this mixture was added 0.1 ml of a solution of sodium chromate ( $13.5 \mu\text{g ml}^{-1}$  in phosphate-buffered saline). After incubation at 37 °C for 1 h, the organisms were washed repeatedly (usually five times) until the count rate in the supernatant had reached an acceptable level (less than 5% of the label in the protozoa). The organisms were then made up to  $10^5$  per ml. Tissue culture Medium



**Table 1** Isotope release from  $^{99m}\text{Tc}$ -pertechnetate labelled *T. dionisii* in the presence of antibody and normal lymphoid cells

Experiment	Time (h)	Dilution of antibody			Lymphocytes alone	Medium alone
		1/10	1/100	1/1,000		
1	3	70.6	61.7	59.9	26.7	17.0
	6	76.9	73.9	67.5	35.0	17.0
2	3	59.7	59.9	48.8	17.8	12.3
	6	72.5	60.0	58.5	16.5	16.3
3	3	47.6	—	26.5	19.8	12.2
	6	49.9	—	26.8	18.2	17.0

Controls: normal rabbit serum alone and antiserum to *T. dionisii* alone gave isotope release not significantly different from that in the presence of medium. Normal rabbit serum with lymphoid cells gave isotope release not significantly different from that of lymphoid cells alone.

Summary of statistical analysis. Effect of antibody treatment very highly significant in all experiments ( $P < 0.05\%$ ). Experiments 1 and 2:  $F = 469$ , d.f. 4 and 90; limiting value for  $F = 5.6$ ; experiment 3:  $F = 83$ , d.f. 3 and 54; limiting value of  $F = 7.01$ . Log<sub>e</sub> least significant difference for comparison of means up to 0.128 for experiment 1 (corresponding to differences of 4% at 30% isotope release and 8.2% at 60%), up to 0.164 for experiment 2 (corresponding to 5.3% at 30% and 10.6% at 60%) and up to 0.174 for experiment 3 (corresponding to 3.8% at 20% and 9.5% at 50%).

199 with 10% foetal calf serum (previously heated at 56 °C for 2 h) was used throughout. Normal human peripheral blood lymphoid cells were prepared by the methocel/carbonyl iron method from defibrinated blood. Rabbit antiserum to *T. dionisii* prepared by three injections ( $10^7$  organisms in Freund's complete adjuvant intramuscularly, subcutaneously; and  $10^7$  sonicated organisms intravenously) at 14-d intervals, and collected 12 d after the last injection, was heated to 56 °C to destroy complement activity, and diluted in medium. Samples of 100  $\mu\text{l}$  of labelled *T. dionisii* ( $10^4$  organisms), 100  $\mu\text{l}$  of diluted rabbit antiserum and 100  $\mu\text{l}$  of human lymphoid cells ( $2 \times 10^5$  cells) were mixed in small plastic tubes and incubated at 37 °C for up to 6 h. After this time half of the supernatant was removed from the tube and counted in a sodium iodide crystal scintillation counter together with the tube containing the remainder of the supernatant and the remaining organisms, so that the percentage of isotope released could be calculated. All experiments were subjected to statistical analysis by analysis of variance, and differences between means of groups of tubes were tested for significance by Duncan's multiple range test.

Table 1 shows that normal human lymphocytes in the presence of dilutions of antibody as high as 1/1,000 cause considerable isotope release from the parasites. Lymphocytes alone cause more isotope release than the spontaneous leakage from organisms incubated in medium as also occurs when human target cells are incubated in the presence of human lymphoid cells, and its significance is not understood.

Figure 1 shows that significant isotope release occurs within 1 h and progresses to completion at about 6 h. Isotope release in the presence of lymphocytes alone also increases, though more slowly. Spontaneous leakage of isotope from organisms incubated in medium alone remains fairly constant between 1 and 6 h.

It would clearly be premature to claim that this demonstration of antibody-dependent lymphocyte-mediated cytotoxicity of *T. dionisii* establishes its role in immunity against protozoal parasites. Nevertheless, the requirements for very small amounts of antibody, its dependence on a ubiquitous normal cell, and the rapidity of its action at least make this form of cytotoxicity a candidate which may have an important role in limiting the spread of intracellular parasites from one cell to another.

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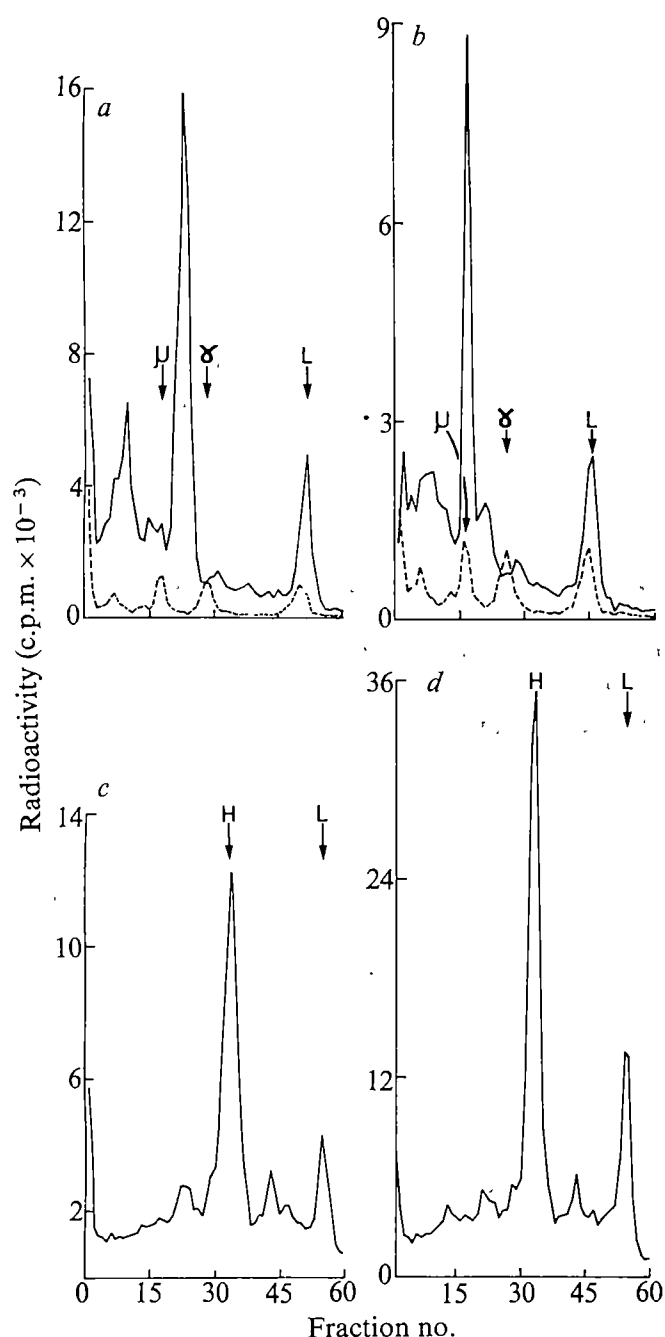
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## Preparation and characterisation of an antiserum to the mouse candidate for immunoglobulin D

ANALYSIS of mouse B-lymphocyte surface immunoglobulin (Ig) by isotopic labelling techniques reveals little, if any, IgA or IgG. Instead, the surface Ig consists of monomeric IgM and an Ig similar in physicochemical properties to human IgD<sup>1-3</sup>. In the absence of sequence comparison between human IgD and its assumed mouse counterpart, this assignment is tentative. With this proviso, however, and for the sake of clarity in presentation, we refer to this immunoglobulin as IgD. During development, IgM-bearing cells are the first to arise and the ratio of IgM-IgD determined with labelled adult lymphocytes is higher in the spleen than in lymph nodes or Peyer's patches<sup>1-4</sup>. These findings, however, give the total yield of the two Ig classes and not their distribution on individual lymphocytes. On the basis of the absence of immunoglobulins other than IgM or IgD on the surface of B lymphocytes, we indicated the presence of cells bearing IgM or IgD, or IgM and IgD in mouse spleen cell suspensions<sup>5,6</sup>. In these experiments, IgM was first capped with rhodamine-labelled anti- $\mu$  and subsequent ring staining with fluorescein-labelled anti-Fab in the presence of sodium azide was taken as evidence for the presence of IgD. We report here the preparation of an antibody specific for mouse IgD, and its use in the characterisation of mouse B-lymphocyte surface Ig.

Plasma cell membranes were prepared from the spleens of 1,750 BALB/c mice (wet weight 160 g) to yield 130 mg of protein. The membranes were dissolved in 25 ml of phosphate-buffered saline (PBS) containing 1% (w/v) Nonidet P-40 and 1 mM phenylmethylsulphonyl fluoride, centrifuged at  $10^5g$  for 3 h, and then passed successively through three columns of Sepharose 4B: (1) normal rabbit Ig (6.0 mg)-Sepharose (3.0 ml); (2) rabbit anti-mouse  $\mu$  (3.6 mg)-Sepharose (1.8 ml); (3) rabbit anti-mouse Fab (5 mg)-Sepharose (2.5 ml). The rabbit anti-mouse  $\mu$  was prepared by applying a specific rabbit anti- $\mu$  serum to IgM ( $\lambda^1$ ) (MOPC 104E)-Sepharose, and then eluting the antibody with molar acetic acid. Anti-Fab was prepared similarly and was a mixture of the following acid eluates: rabbit anti-whole IgG<sub>1</sub>(K)(MPC 21) retained by IgG<sub>2a</sub>(K)(Adj PC5)-Sepharose, rabbit anti-whole IgG<sub>2a</sub>(K)(Adj PC5) retained by IgG<sub>1</sub>(K)(MPC 21)-Sepharose and rabbit anti-whole IgM(K)(TEPC 183) retained by IgG<sub>2a</sub>(K)(Adj PC5)-Sepharose. Purified antibody was coupled to Sepharose<sup>8</sup> to give high capacity immunoabsorbants. After passage of the sample, the columns were washed with phosphate-buffered



**Fig. 1** Surface immunoglobulin of murine splenic lymphocytes. Spleen cell suspensions of 3–6-month-old, specific pathogen-free, female CBA mice were labelled with  $^{125}\text{I}$  by the lactoperoxidase-catalysed procedure<sup>18</sup>, washed once in ice-cold PBS and then lysed for 10 min at 0 °C in 1% (w/v) Nonidet P-40 in PBS containing 1 mM phenylmethylsulphonyl fluoride and 100 mM recrystallised iodoacetamide. The centrifuged lysate ( $30,000g \times 15$  min) was passed over Sephadex G-25 equilibrated with the solution used for lysis of the cells. Labelled IgD was precipitated at 0 °C by addition of rabbit antibody (10  $\mu\text{l}$ ) and a goat anti-rabbit Ig (100  $\mu\text{l}$ ). The precipitate was removed by precipitation and the supernatant was further reacted with rabbit anti-mouse  $\mu$  chain (10  $\mu\text{l}$ ) and goat anti-rabbit IgG (100  $\mu\text{l}$ ). Both precipitates were washed three times with ice cold 0.5% (w/v) Nonidet P-40 in PBS, once with 50 mM sodium phosphate, pH 7.0, and then dissolved in 50 mM sodium phosphate, pH 7.0, containing 2% (w/v) sodium dodecyl sulphate (SDS) and 1 mM dithiothreitol. The precipitates were heated at 100 °C for 10 min, and then iodoacetamide was added to 100 mM. Internal  $^{125}\text{I}$ -labelled<sup>19</sup> markers were added to the labelled cell surface Ig samples, which were then resolved by SDS-polyacrylamide gel electrophoresis<sup>1</sup>. After electrophoresis the gels were sliced into 1-mm segments and radioactivity was determined. Values were corrected for cross-channel spill and plotted with the top of the gel on the left hand side of the figure. Reduced cell surface Ig precipitated with the anti-IgD (a and c) and subsequently anti- $\mu$  (b and d) were electrophoresed on 7.5% (a and b) and 4.2% (c and d) polyacrylamide gels. —,  $^{125}\text{I}$  incorporated into cell surface Ig; . . . ,  $^{125}\text{I}$ -labelled internal marker.

1–4) A similar profile resulted when 100  $\mu\text{l}$  of normal mouse serum was incubated with 10  $\mu\text{l}$  of the rabbit antiserum (30 min room temperature) before addition of the radioactive sample. Thus circulating IgD is likely to be present in very small amounts in the mouse. Recovery of IgM was possible from the supernatant of the first precipitation by addition of anti- $\mu$  (Fig. 1b). The radioactive material which runs before and after the  $\mu$  and  $\delta$  chains also occurs in non-specific precipitates. Furthermore, the higher molecular weight material largely disappears when less concentrated polyacrylamide gels are used (Fig. 1c and d), and thus may represent an aggregation artefact. The disadvantage of the less concentrated polyacrylamide gels is that there is no resolution between the  $\mu$  and  $\delta$  chains. When unreduced samples of cell surface Ig were electrophoresed on 4.2% (w/v) polyacrylamide gels, the material then coincided with a disulphide-linked 7S IgM internal marker<sup>1</sup>.

On the basis of these results, we were therefore confident that our antiserum was specific for mouse IgD, and thus turned to studies using fluorescent reagents prepared by DEAE chromatography<sup>10</sup> to stain live cells<sup>11</sup>. With 1–2-week-old CBA mouse splenocytes, we were unable to find significant numbers of cells reacting with the anti-IgD, whereas anti-mouse  $\mu$  chain revealed 8–14% of the cells as positive. Cells bearing IgD were, however, readily apparent in the spleens of 6-week-old mice (~20% total lymphocytes). Absorption of the antiserum with foetal liver cells ( $3.6 \times 10^{10}$  cells per ml of serum) did not remove its capacity to react with spleen cells, whereas absorption with adult spleen cells did ( $1.9 \times 10^{10}$  cells per ml of serum). The finding that IgM precedes IgD in ontogeny<sup>1,3</sup> is therefore confirmed.

We next determined the distribution of IgM and IgD on lymphocytes from various lymphoid organs (Table 1). Single staining was done by the indirect procedure. Double staining was achieved by capping IgD with a fluorescein-labelled system followed by staining for IgM with a rhodamine-labelled rabbit anti-mouse  $\mu$  chain under non-capping conditions. From the single staining results it is immediately clear that IgD constitutes the major cell surface immunoglobulin of lymph nodes and Peyer's patches, as would be suggested by biochemical investigations<sup>1–4</sup>. Double staining of lymphocytes from these organs not surprisingly revealed most of the Ig-positive cells as having only IgD, although some doubles (IgM and IgD) and occasional cells positive for IgM alone were also present. In the spleen, however,

saline (PBS), emulsified in complete Freund's adjuvant and injected into rabbits<sup>9</sup>. Each column was divided between two rabbits. Serum from a rabbit receiving the third column, the anti-Fab, was passed over IgG<sub>2a</sub>(K)(Adj PC5)-Sephadex and then IgM(K)(TEPC 183)-Sephadex. The absorbed serum was tested by radioimmunoassay<sup>5</sup> and found to be unreactive with  $^{125}\text{I}$ -labelled IgM(K)(TEPC 183), IgM( $\lambda_1$ )(MOPC 104E), IgA( $\lambda_2$ )(MOPC 315), IgG<sub>1</sub>(K)(MPC 21), IgG<sub>2a</sub>(K)(Adj PC5), IgG<sub>2b</sub>(K)(MOPC 195), IgG<sub>3</sub>(FLOPC 21),  $\lambda$  chains (RPC 20), K chain (MOPC 41) and mouse  $\alpha_2$ -macroglobulin. The radioimmunoassay will detect less than 1  $\mu\text{g}$  of antibody in 1 ml of serum.

When used in conjunction with rhodamine-labelled goat anti-rabbit Ig (ref 10), the absorbed antiserum stained<sup>11</sup> about 20% of CBA strain spleen cells, but not thymocytes from CBA, C57BL, DBA/2, C3H, AKR and BALB/c mice. By this test, the antiserum was also negative for peripheral T cells prepared by the nylon wool method<sup>12</sup>.

We then evaluated the antiserum by testing with  $^{125}\text{I}$ -surface labelled spleen cells as described before<sup>1</sup>, and found the only Ig precipitated corresponded to IgD (Fig. 1a, refs

Table 1 Frequency of IgM and IgD-bearing cells in mouse lymphoid tissue

	Organ	Anti-Ig	Single staining		$\mu$	Double staining	
			Anti- $\mu$	Anti- $\delta$		$\mu+\delta$	$\delta$
Experiment 1	Peripheral nodes	14.9	3.1	10.5	0.7	1.5	8.1
	Mesenteric nodes	14.4	4.1	10.5	1.1	1.8	7.2
	Peyer's patches	23.1	8.5	26.8	1.6	8.5	19.3
	Spleen	35.6	19.1	23.8	12.1	13.8	12.6
Experiment 2	Peripheral nodes	15.6	3.1	9.1	0.5	2.7	11.1
	Mesenteric nodes	14.7	3.6	12.6	0.8	2.8	11.6
	Peyer's patches	23.2	9.4	22.5	0.8	8.2	14.0
	Spleen	32.2	17.8	25.9	8.7	11.9	14.3

Cell suspensions were prepared from 7-month-old, specific pathogen-free, female CBA mice by teasing the organs with veronal-buffered saline (Oxoid), containing bovine serum albumin (1 mg ml<sup>-1</sup>) (VBS-BSA), and the living cells were stained and prepared for examination as described by Raff<sup>11</sup>. Peripheral nodes collected were superficial cervicals, axillary, brachial and inguinal. For single staining the cells were incubated for 20 min at room temperature with rabbit antiserum, washed three times with VBS-BSA and then suspended in rhodamine-labelled goat anti-rabbit IgG (20 min, room temperature). Samples were washed, and then mounted in 50% (w/v) glycerol in (PBS)-0.03 M sodium azide. For double staining, cells were treated with anti-mouse IgD and fluorescein-labelled goat anti-rabbit IgG as described above. The two layers were essential to ensure capping of all the IgD positive cells. The cells were then incubated in the cold for 20 min with rhodamine-labelled rabbit anti-mouse  $\mu$  chain and with sodium azide at 0.03 M. After washing with cold VBS-BSA-0.03 M sodium azide the cells were mounted and examined under ultraviolet light with a Leitz Orthoplan research microscope fitted with Ploem illumination. At least 500 lymphocytes were assessed for staining and the results are given as percentages of total lymphoid cells observed. All reagents were centrifuged (50,000g, 60 min) on the day of use. On the basis of the double staining protocol, lymphocytes could be classified into three groups, those with green caps (IgD positive), those with peripheral red staining (IgM positive) and those with green caps and red rings (IgM and IgD positive). The rabbit anti-mouse Ig was prepared by immunisation of rabbits with the Fab portion of myeloma protein Adj PC5 ( $\gamma_{2a}$ K) (ref. 20) as described before<sup>9</sup> and was polyspecific, reacting against IgM, IgG<sub>1</sub>, IgG<sub>2a</sub>, IgG<sub>2b</sub>, IgG<sub>3</sub> and IgA (but not other serum proteins) on Ouchterlony analysis. Rabbit anti-mouse  $\mu$  chain was prepared by immunisation with myeloma protein MOPC 104E ( $\mu\lambda_1$ )<sup>9</sup> and absorbing the serum with a 7S fraction of normal mouse serum coupled to Sepharose 4-B. The resulting serum precipitated IgM on Ouchterlony analysis, but did not react with any other mouse Ig class when tested by radioimmunoassay against <sup>125</sup>I-labelled immunoglobulins (see the text and ref. 5). Preparation of rabbit anti-mouse IgD is described in the text. Fluorochrome conjugates were prepared using DEAE-purified Ig fractions, and conjugates with fluorochrome: protein ratios of 1.5–2.5 were selected by chromatography on DEAE<sup>10</sup>.

all three categories of cells were represented in similar proportions.

Our findings in the mouse therefore mirror those in the human<sup>13,14</sup>. In the mouse, there are fewer cells bearing both IgM and IgD, with correspondingly higher numbers of cells with exclusively IgM or IgD. These studies in the human, however, have been made with peripheral blood and so the comparison is not necessarily valid. The biological significance of these subpopulations of B cells should now be open to investigation. Since IgM precedes IgD in ontogeny, we favour the idea that in B-lymphocyte maturation there is differentiation from cells expressing only IgM to those expressing only IgD (ref. 5). An intervening cell type expresses both Ig classes. Thus the B lymphocytes of lymph nodes and Peyer's patches would in the main constitute a more mature population, possibly enriched with memory cells and precursors of cells destined to secrete IgG and IgA. The finding that IgA precursor cells of rabbit Peyer's patches are IgM and IgA negative, but certainly do have surface immunoglobulin<sup>15</sup>, suggests that these cells bear the rabbit equivalent of IgD.

The simultaneous expression of two Ig classes on the cell surface has implications for "switch" and V-C gene integration mechanisms. For example, the two constant region genes for  $\mu$  and  $\delta$  chains are known to share the same V region<sup>16</sup>, and if this reflects the simultaneous presence of two integrated genes (that is VH-C $\mu$  and VH-C $\delta$ ), then a copy-choice mechanism for V-C gene integration<sup>17</sup> is suggested.

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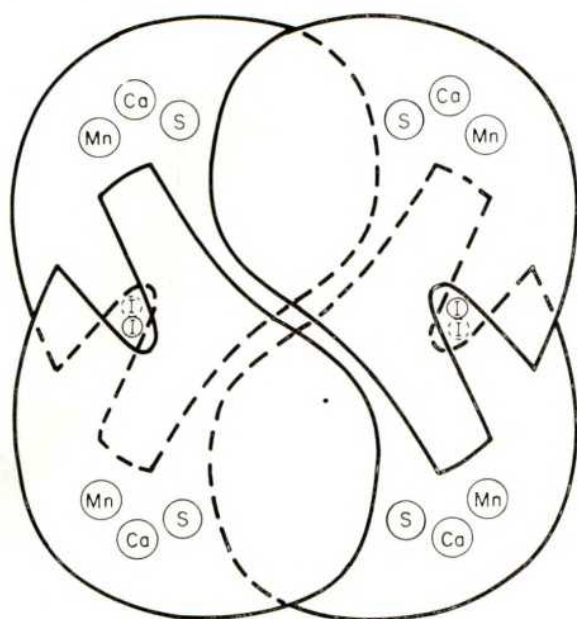
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## New evidence on the location of the saccharide-binding site of concanavalin A

THE biological activities<sup>1–4</sup> of the mitogenic lectin concanavalin A (con A) depend on the binding of the protein to receptors on the cell surface, and this binding can be inhibited by specific saccharides related to D-mannose and D-glucose<sup>5</sup>. Knowledge of the location and nature of the saccharide-binding site of con A would therefore contribute greatly to understanding the molecular basis of its biological activities. Although the complete amino acid sequence<sup>6–8</sup> and three-dimensional structure<sup>9–11</sup> of con A are known, there has been conflicting evidence on the location of the carbohydrate-binding site. Crystallographic studies with saccharides labelled with heavy atoms indicated that it was in a deep cavity in the molecule, more than 20 Å from the Mn<sup>2+</sup> and Ca<sup>2+</sup> ions<sup>9,12</sup> (Fig. 1). Subsequent magnetic resonance measurements<sup>13–15</sup> as well as crystallographic considerations<sup>16</sup> suggested that the site was 10–12 Å from the metals, and led to the hypothesis that the previously studied saccharides labelled with heavy atoms (for example, o-iodo-phenyl-β-D-glucopyranoside (β-IPG)) were bound by their hydrophobic aglycones, rather than by their saccharide moieties<sup>9,17</sup>.

High concentrations of inhibitory saccharides crack or dissolve con A crystals of the kind used in the structure determination, precluding crystallographic observation of the





**Fig. 1** Schematic representation of the con A tetramer. Each subunit is approximately  $42 \times 40 \times 39$  Å, and the tetrameric complex possesses exact 222 symmetry. The manganese and calcium sites are indicated by Mn and Ca, respectively. The saccharide-binding site near the metals is indicated by S and the  $\beta$ -IPG-binding site in the cavity by I.

carbohydrate-binding site. We have therefore adopted several different approaches to locate the site. We describe here the results of three such experiments: examination of the structure of an insolubilised crystalline con A-saccharide complex, investigation of the structure of demetallised inactive con A, and preparation of a new crystal form of con A containing bound saccharides.

Although specifically bound sugars disrupt con A crystals of the type used in the original structure determination, we have carried out diffraction studies of carbohydrate binding using these crystals. Con A crystals, prepared as described before<sup>9</sup>, were treated with 1% glutaraldehyde for 1 h at room temperature. Addition of specific saccharides to these cross-linked crystals caused considerable changes in the diffraction pattern and a reduction of the limiting resolution from 2 Å to 3.5 Å (Fig. 2). These changes were reversed by soaking the crystals in saccharide-free buffer, and non-binding carbohydrates, such as methyl- $\alpha$ -D-galactopyranoside, did not produce such changes, suggesting that the alteration of the diffraction is caused by non-covalently bound specific saccharide.

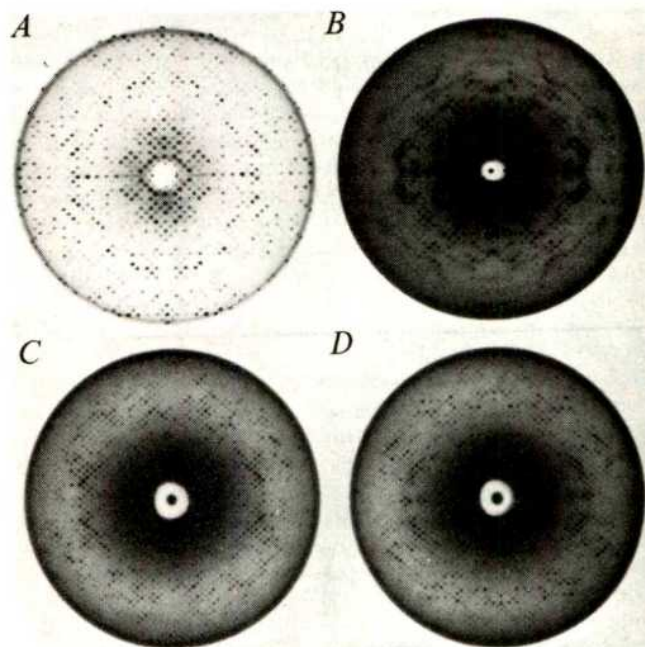
**Table 1** Comparison of locations of heavy-metal substituents in con A and its complex with 2-deoxy-2-iodo-methyl- $\alpha$ -D-mannopyranoside

Heavy-metal site*	Coordinates in native con A			Coordinates in the complex		
	x	y	z	x	y	z
Pb1	-13.67	-11.43	18.10	-14.19	-11.79	17.81
Pb2	-8.90	-22.59	11.16	-10.20	-22.10	11.37
Pt1	5.30	-0.35	3.66	5.57	1.04	4.42
Sm1	-13.58	-11.43	18.16	-13.36	-11.70	18.20
Sm3	-8.90	-23.29	11.67	-9.18	-23.05	12.41
II	0.45	-12.47	12.42	0.74	-12.91	13.32

\*Coordinates are in Å referred to an origin at the 222 point of each protein system. Overall figure of merit for the 2-iodo- $\alpha$ -methylmannoside-con A phasing calculation is 0.714 for 1,894 reflections. Heavy-metal sites are named as in ref. 9, except II refers to an iodophenol derivative of the saccharide complex.

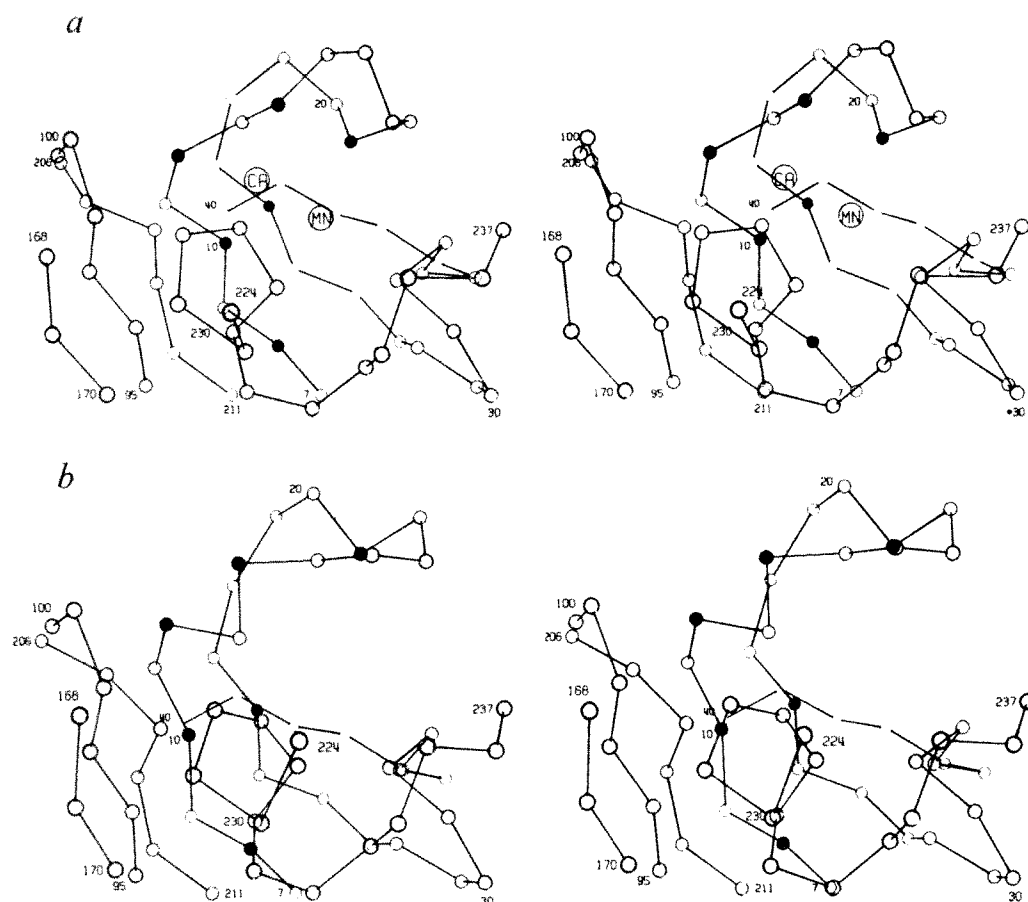
To avoid potential nonspecific binding to a hydrophobic aglycone, 2-deoxy-2-iodo-methyl- $\alpha$ -D-mannopyranoside<sup>18</sup>, an inhibitor labelled with heavy atoms, was used to form a con A-saccharide complex. Attempts to solve the structure of this complex with phases from native con A produced electron density maps that we could not interpret. Accordingly, new phases were calculated by multiple isomorphous replacement methods, and refined by electron density modification<sup>19</sup>. The molecular packing is like that of native con A, as indicated by the close similarity of the heavy atom positions in the two proteins (Table 1). In addition, a rotation function<sup>20</sup> calculated between native and saccharide-treated con A has its highest maximum at nearly zero net rotation.

Nearly all the polypeptide chain can be traced in the electron density map, and preliminary interpretation suggests that significant local differences in conformation are induced by carbohydrate binding. The positions of chain segments 95-105 and 195-207 relative to the rest of the molecule are altered, resulting in a disruption of the  $\beta$ -structure in this region. The entire metal-binding region seems to have moved 4-6 Å with respect to the rest of the molecule (Fig. 3). Local changes in this region cannot yet be described in detail, but there seem to be related changes in residues 33-45 and the top two chains of the back  $\beta$ -structure<sup>9</sup>. To locate the bound sugar molecule, a difference map between the complexes with the iodo sugar and its chloro analogue was calculated. The highest peak in this map was approximately 13 Å from the Mn<sup>2+</sup> ion, at 0.74, 0.24, 0.42, which we interpret to be near to residues Gly 227 and Arg 228, in the metal-binding region of the molecule. Assuming that this peak represents the iodine of a bound saccharide molecule, the residues that may participate in con A-saccharide interactions are 14-16, 97-99, 168-169, 207-208, 224-228 and 235-237. These residues surround a shallow pocket near the top of the molecule (Figs 1 and 3)



**Fig. 2** A,  $hk0$  zone of the native con A diffraction pattern. Reflections at the edge of the circle correspond to Bragg spacings of 2.8 Å. The space group is I222 with  $a = 89.91$ ,  $b = 87.23$ ,  $c = 63.07$  Å. B,  $hk0$  zone of the cross-linked 2-iodo- $\alpha$ -methylmannoside complex of con A. Space group I222, with  $a = 92.76$ ,  $b = 86.66$ ,  $c = 64.99$  Å. C,  $hk0$  zone of triclinal con A crystallised from 20% saturated ammonium sulphate, pH 6.8. For comparison with D, a centred cell has been chosen, space group C1,  $a = 119.04$ ,  $b = 103.23$ ,  $c = 132.09$  Å,  $\alpha = 89^\circ 50'$ ,  $\beta = 94^\circ 17'$ ,  $\gamma = 85^\circ 03'$ . D,  $hk0$  zone of a con A crystal grown as in (C) and then treated with 50 mM  $\alpha$ -methyl-D-glucopyranoside in 50% saturated ammonium sulphate. Space group C222<sub>1</sub>, with  $a = 118.09$ ,  $b = 103.54$ ,  $c = 251.8$  Å.





**Fig. 3** *a*, Stereo drawing of the polypeptide backbone in the metal-binding region of native con A. ○, Alpha carbon atoms; ●, residues which serve as metal-binding ligands; CA and MN, calcium and manganese ions, respectively. *b*, The corresponding region of demetallised con A. The opening up of the polypeptide chain around the vacant metal-binding site is visible at the upper right.

and several of them participate in intermolecular interactions in the crystal. Disruption of these lattice interactions on saccharide binding may account for the difficulty of observing specifically bound saccharides in crystals of the type used in the original structure determination. The difference map shows no significantly large peak in the molecular cavity, suggesting that the binding site is elsewhere. This interpretation requires that the binding region in the cavity be a nonspecific site, active only in crystalline con A, inasmuch as in solution, hydrophobic sugars, such as  $\beta$ -IPG, are bound only at the specific site<sup>9</sup>.

The location of the site near the metal-binding region is supported by a study of the structure of demetallised con A. Removal of the bound  $Mn^{2+}$  and  $Ca^{2+}$  ions from native con A causes loss of saccharide-binding activity<sup>21-23</sup>, suggesting that the saccharide-binding site is associated with a part of the con A structure that changes significantly with demetallisation. Demetallised con A was prepared<sup>23,24</sup> and crystallised<sup>25</sup>, and diffraction data to a resolution of 2.8 Å were collected as before<sup>9</sup>. The native and demetallised structures were aligned using molecular replacement methods. In separate experiments, structure factors were calculated for a native con A monomer or dimer in a triclinic cell with the demetallised unit cell dimensions. Complete rotational<sup>20</sup> and translational<sup>26</sup> searches indicated that the demetallised molecule was related to the analogous native con A dimer by a rotation of 7.5° about an axis near  $x$ , in agreement with earlier results<sup>25</sup>, ( $\alpha = 273.8^\circ$ ,  $\beta = 7.5^\circ$ ,  $\gamma = 87.7^\circ$  using Crowther's notation<sup>20</sup>) and shifted slightly along  $z$  (translation = -0.0004, -0.0002, 0.0106 in the demetallised cell), the dimer moving as a single rigid body. (Details of the molecular replacement calculations will be published elsewhere.)

Structure factors calculated for this model, excluding residues in the metal binding region (residues 7-25), showed excellent agreement with the observed data for the demetallised molecule (conventional crystallographic  $R = 42.6\%$ , as compared with 40.4% for the final native con A structure). Difference maps

were calculated with coefficients

$$[(2|F_o| - |F_c|) \exp(i\alpha_c)]$$

and

$$[(|F_o| - |F_c|) \exp(i\alpha_c)]$$

where  $F_o$  are observed demetallised structure factors,  $F_c$  are model structure factors, and  $\alpha_c$  are model phases. Examination of these maps allowed construction of a model of the metal-binding region and comparison of the two structures. The largest differences between the two structures were confined to the metal-binding region, the most significant being an opening of the tightly folded loop (residues 11-23) above the metal atoms, and a rearrangement of the nearby chains involving residues 99, 209, 228 and 237 (Fig. 3). The remainder of the molecule seems to be very similar in the two structures, with most of the changes being small (<1 Å) motions of the backbone or rotations of side chains. In particular, the folding in the region of the molecular cavity is almost identical. This localisation of significant structural differences between active con A and inactive demetallised con A strongly supports the hypothesis that the saccharide-binding site is in the vicinity of the metal-binding residues, approximately 13 Å from the  $Mn^{2+}$  ion, and not in the deep cavity (Fig. 1).

Definitive crystallographic proof of the location of the saccharide-binding site and its detailed characterisation must await the determination at high resolution of the structure of a con A-saccharide complex. To this end, we have prepared a new crystal form of con A that can bind specific sugars without loss of the high resolution diffraction pattern (Fig. 2). The striking similarity between diffraction from this crystal form and that from native con A suggests that solution of the structure by molecular replacement methods will be feasible. It is likely that this determination will confirm the interpretation suggested by the experiments described here that the saccharide-binding site is directly associated with the metal-binding

region of con A. This close association allows a simple explanation of the dependence of saccharide-binding activity on the presence of the metal ions, as the metals may directly stabilise the saccharide-binding residues in their active conformation.

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## Concanavalin A blocks desensitisation of glutamate receptors on insect muscle fibres

CONCAVALIN A (con A), a tetrameric lectin which binds to specific carbohydrate residues<sup>1</sup>, has been used in the purification of a glutamate-binding glycoprotein isolated from rat brain synaptosomes<sup>2</sup>, and has been shown to alter the isotherm describing the binding of  $\alpha$ -bungarotoxin to acetylcholine receptors on rat skeletal muscle<sup>3</sup>. Complementary electrophysiological studies on the effect of con A on intact receptor-bearing membranes are, as yet, lacking. We present here evidence that con A abolishes the desensitisation normally observed during repeated iontophoretic application of L-glutamate, the putative excitatory transmitter, to two populations of L-glutamate receptors on locust skeletal muscle fibres.

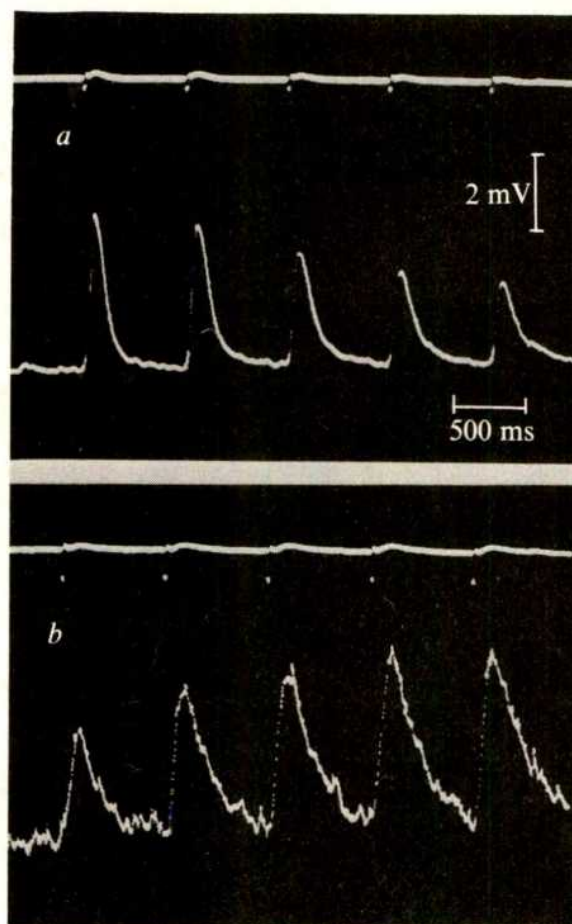
Iontophoresis of L-glutamate (pH 7.0, 1 M) through high resistance (120-300 M $\Omega$  micropipettes) is known to produce three types of response on innervated<sup>4,5</sup>, as opposed to denervated<sup>6</sup>, locust extensor tibiae muscle fibres. When the tip of the glutamate electrode is within a few micrometres of a neuromuscular junction, a rapid transient depolarisation of rise time 10-50 ms and peak sensitivity  $\sim 100$  mV nC<sup>-1</sup> is seen. This event is associated with the activation of excitatory junctional glutamate receptors<sup>4,5</sup>.

These receptors exhibit desensitisation at glutamate pulse repetition rates  $\geq 0.7$  Hz (ref. 5 and Fig. 1a).

Iontophoresis of 1-5 nC of glutamate on to the extrajunctional membrane of locust muscle fibres evokes a biphasic response due to simultaneous activation of two populations of receptors designated as D-type receptors and H-type receptors, which are associated with ionophores for cations (D) and ionophores for Cl<sup>-</sup> (H)<sup>7,8</sup>. The D and H receptors occur evenly over the entire extrajunctional membrane, their sensitivity to L-glutamate iontophoresis being 0.2-0.5 mV nC<sup>-1</sup> (ref. 6), and both types are very readily desensitised by repeated pulses of iontophoretically-applied glutamate (Fig. 2a, b). Indeed, the diffusion of glutamate which occurs from drug-filled micropipettes of resistance  $< 100$  M $\Omega$  is sufficient in itself to inactivate these receptors<sup>8</sup>.

Con A (Sigma, Grade IV), was dissolved in locust saline of composition 200 mM NaCl, 10 mM KCl, 2 mM CaCl<sub>2</sub>, 4 mM NaH<sub>2</sub>PO<sub>4</sub>, 6 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.8, and applied topically to the preparations at a concentration of 10<sup>-7</sup> M. No change in membrane potential, input resistance or of frequency of spontaneous miniature potentials was observed. Within 0.5-1 h at 20-22 °C, the depolarising component of the biphasic extrajunctional response was no longer abolished by repeated pulses of glutamate. The H receptors, however, retained their normal rate of inactivation in these conditions (Fig. 3a-c). In sensitivity (0.2-0.5 mV nC<sup>-1</sup>), rise time (40-60 ms) and homogeneous distribution over the extrajunctional membrane, the glutamate-

Fig. 1 a, Iontophoresis of 1-nC glutamate pulses at 720 ms intervals on to an excitatory junction of a muscle bathed in 10<sup>-7</sup> M con A for 5 h. Responses showed desensitisation at a rate typical of junctional receptors in control fibres. b, Fibre exposed to 10<sup>-6</sup> M con A for 1 h. Responses now exhibited simultaneous potentiation and summation to 1-nC glutamate pulses delivered at the same frequency as in a. Note high frequency of spontaneous miniature end plate potentials.





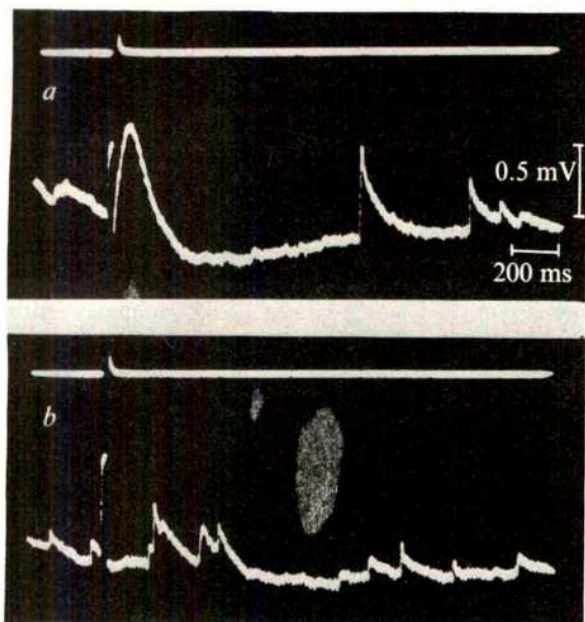


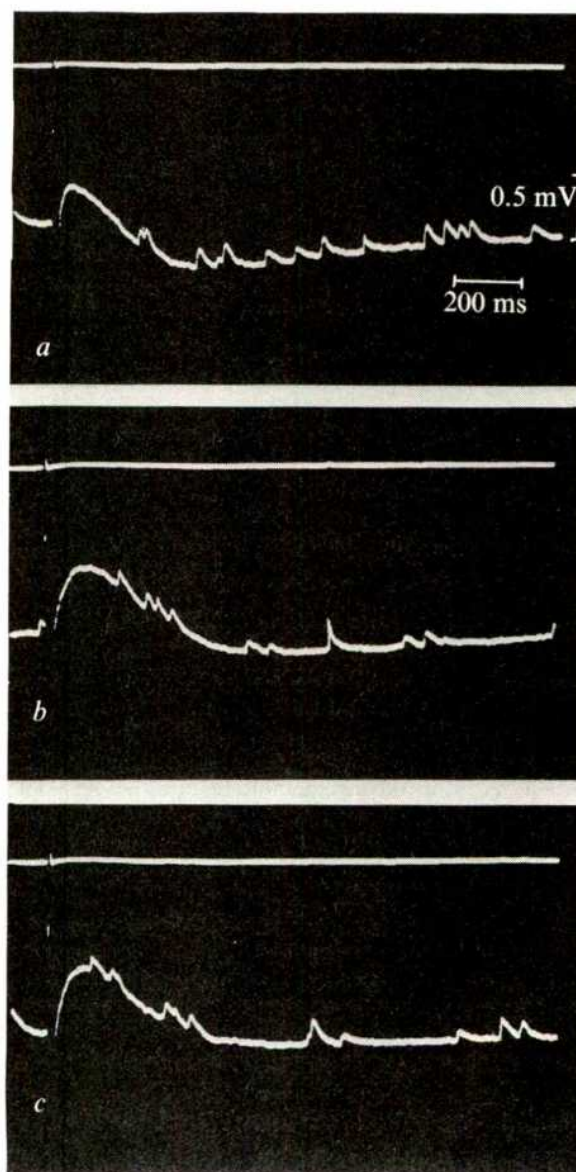
Fig. 2 *a*, Biphasic (D-H) response (lower trace) evoked by iontophoresis of a 5-nC pulse (upper trace) of L-glutamate on to the extrajunctional membrane of a locust muscle fibre. *b*, A second pulse, delivered 4 s later, produced only a small hyperpolarisation, indicating that both D- and H-receptor populations had undergone marked desensitisation. Miniature excitatory potentials are seen on the lower traces.

induced depolarisation in the presence of con A resembled closely the normal D response elicited from control preparations. Bathing the nerve-muscle preparation in saline in which  $\text{Na}^+$  was substituted by choline reversibly abolished the D responses both in the presence and absence of con A.

The increased rate of application of an agonist is known to accelerate receptor desensitisation<sup>9</sup>. This phenomenon, also characteristic of both junctional and extrajunctional D receptors in control fibres of locust muscle, was not observed when glutamate was iontophored on to the extrajunctional membrane following exposure to  $10^{-7}$  M con A. When the frequency of glutamate pulses was raised, potentiation of the D potentials occurred (Fig. 4*a*), while at still higher frequencies a summated form of response appeared (Fig. 4*b*), the amplitude of which was stable for pulse trains of  $< 20$  s. This summated D response decayed rapidly to zero on switching off the iontophoretic current. The rise time of the summated D response in con A saline was reduced with increased rate of glutamate application (that is, increased pulse amplitude or frequency), while the amplitude and decay time were elevated by this procedure. Lateral movement of the glutamate electrode had no significant effect on the quantitative or qualitative aspects of this summated depolarisation, thus confirming its extrajunctional origin. The desensitisation of junctional glutamate receptors was not blocked by  $10^{-7}$  M con A. On increasing the concentration of con A to  $10^{-6}$  M, however, iontophoresis of glutamate on to excitatory synaptic sites evoked responses qualitatively similar to those seen on the extrajunctional membrane but of higher sensitivity and circumscribed origin (Fig. 1*b*). Since this concentration of con A also produced a significant rise in miniature potential frequency (see Fig. 1*b*), it is probable that the threshold concentration differential between D and junctional receptors reflects the presence of protective mechanisms at synaptic sites rather than a difference in the susceptibility of the two receptor groups to con A. The effects of con A on both junctional and D-receptor desensitisation could not be reversed by prolonged washing in con A-free saline.

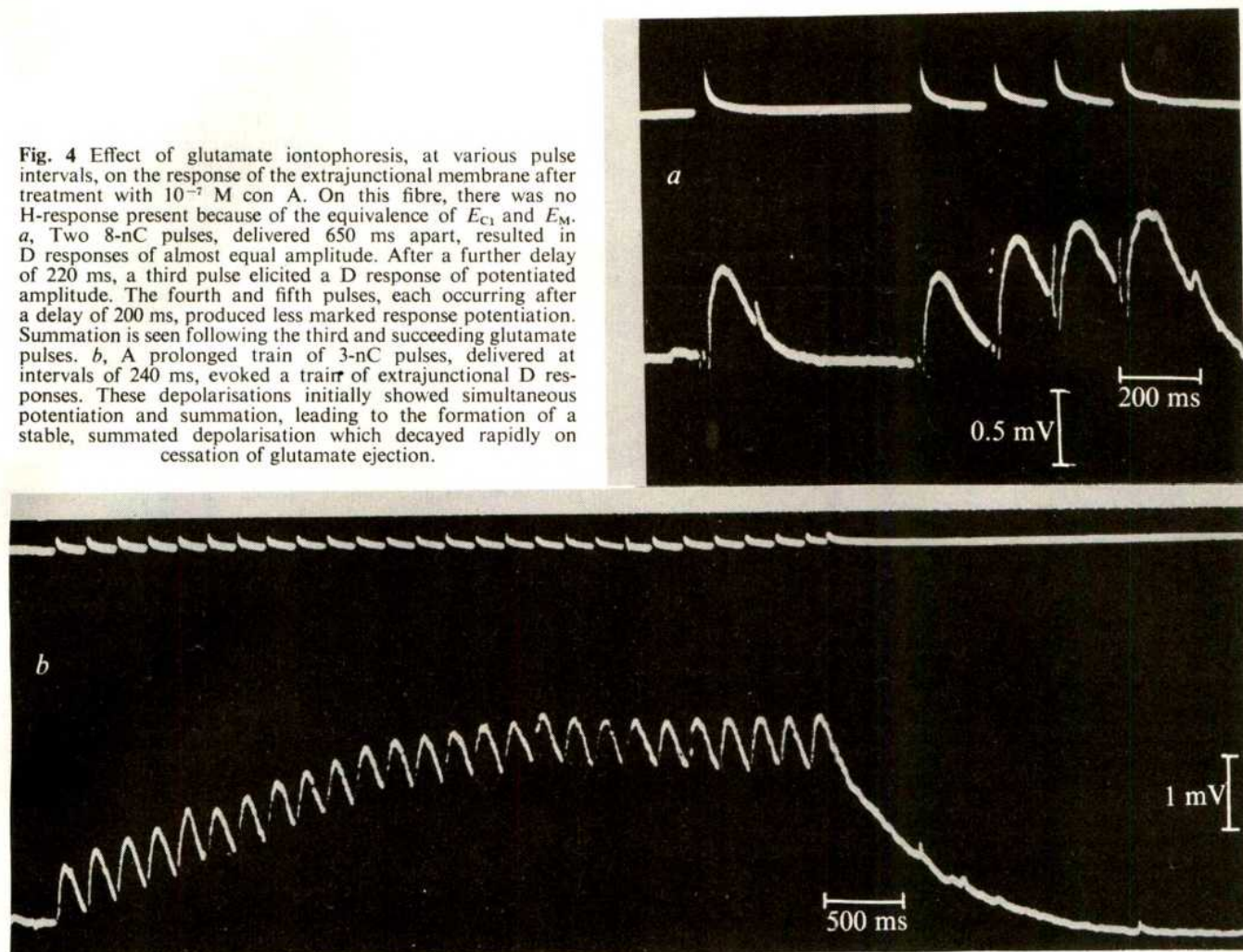
Con A blocks the aggregation of  $\beta$ -lymphocyte immunoglobulin molecules which normally follows on their exposure to specific divalent antibodies<sup>10-12</sup>. This inhibition is believed to be mediated by a submembranous microtubular array which forms as a consequence of the binding of con A to its own glycoprotein receptors<sup>13</sup>. We find it difficult to apply a mechanism of this nature to our observations in view of the normal desensitisation rate of the H receptors in the presence of con A. The H receptor is thought to possess structural features absent from the junctional and D-receptor populations. These presumably account for its activation by DL-isotonic acid<sup>7,8</sup>, a conformationally restricted glutamate analogue, and possibly its association with a  $\text{Cl}^-$  ionophore<sup>14</sup> whose conductance is blocked by picrotoxin. Con A may act by binding directly to junctional and D-receptor molecules, thereby preventing the conformational changes thought necessary for desensitisation to occur<sup>15-17</sup>, but leaving the initial molecular processes, associated with receptor activation and ionophore opening, unaffected. The structural features peculiar

Fig. 3 Iontophoresis of 5-nC pulses of L-glutamate on to the extrajunctional membrane of a fibre bathed in saline containing  $10^{-7}$  M con A for 40 min. *a*, The first pulse elicited a biphasic response; the second and third pulses (*b*, *c*) delivered at 4-s intervals, showed progressive reduction in the hyperpolarising component due to desensitisation of the H receptors. There was no evidence for desensitisation for the D receptors in these conditions.





**Fig. 4** Effect of glutamate iontophoresis, at various pulse intervals, on the response of the extrajunctional membrane after treatment with  $10^{-7}$  M con A. On this fibre, there was no H-response present because of the equivalence of  $E_{Cl}$  and  $E_M$ . *a*, Two 8-nC pulses, delivered 650 ms apart, resulted in D responses of almost equal amplitude. After a further delay of 220 ms, a third pulse elicited a D response of potentiated amplitude. The fourth and fifth pulses, each occurring after a delay of 200 ms, produced less marked response potentiation. Summation is seen following the third and succeeding glutamate pulses. *b*, A prolonged train of 3-nC pulses, delivered at intervals of 240 ms, evoked a train of extrajunctional D responses. These depolarisations initially showed simultaneous potentiation and summation, leading to the formation of a stable, summated depolarisation which decayed rapidly on cessation of glutamate ejection.



to the H receptor may prevent the con A binding step.

It is likely that there are several glycoproteins on the surface of locust muscle membranes to which con A might bind. Perhaps the glutamate receptors on this membrane, like the putative glutamate receptors isolated from rat brain<sup>7</sup>, are glycoproteins although this has not yet been established<sup>18</sup>. It is of interest that the binding of con A to rat brain glycoprotein cannot be reversed on simple washing, a feature shared with the desensitisation characteristics of locust muscle glutamate receptors that we observed. If the receptors on locust muscle are not glycoproteins, then the restriction of the conformational change which possibly leads to desensitisation of these receptors could be the result of con A binding to membrane glycoproteins closely associated with the receptors. Whatever the explanation for the action of con A on insect muscle, this substance will probably prove of great value in the further biochemical characterisation of glutamate receptors from both vertebrate and invertebrate sources. The drug may also provide a means of dissecting the molecular mechanisms underlying receptor desensitisation.

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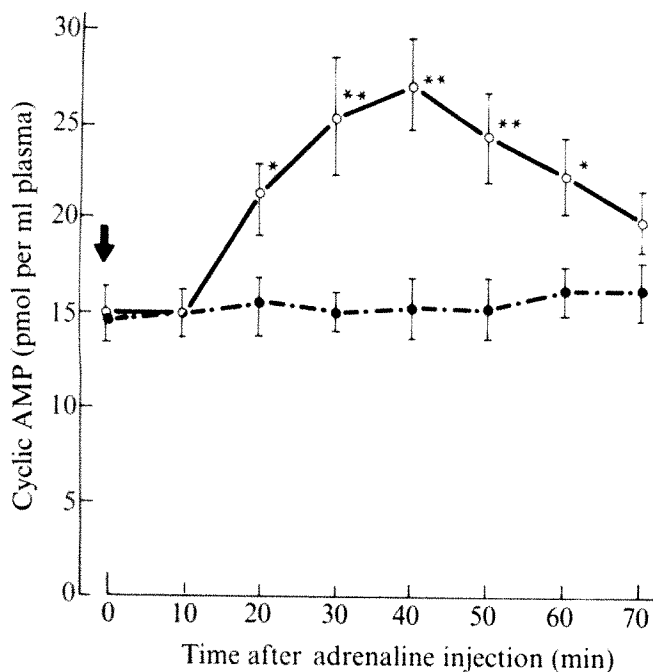
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## Lithium inhibition of adrenaline-stimulated adenylylase in humans

LITHIUM is an effective therapeutic agent in the treatment and prevention of the manic phase of manic-depression<sup>1</sup>, and although known to influence many physiological systems, no specific mechanism has been identified for its efficacy in this illness<sup>2</sup>. One postulated primary site of Li action is as an inhibitor of hormone-stimulated adenylylase<sup>3</sup>. Li inhibits the ADH-sensitive adenylylase in kidney<sup>4,5</sup>, the TSH-sensitive adenylylase in thyroid<sup>6,7</sup>, the prostaglandin-sensitive adenylylase in human platelets<sup>8,9</sup>, and the noradrenaline-sensitive adenylylase in rat brain<sup>10,11</sup>.

It is therefore possible that therapeutic concentrations of Li in man inhibit adenylylase activity. Ball *et al.*<sup>12</sup> have shown that administration of  $\beta$ -adrenergic agonists in





**Fig. 1** The effect of Li on plasma cyclic AMP response to adrenaline administration. Subjects received 0.5 mg adrenaline subcutaneously at time 0 and blood was drawn every 10 min using an indwelling catheter in the antecubital vein. (Subcutaneous adrenaline injection was chosen rather than intravenous injection for greater clinical safety, in spite of the possibility of increased variability.) Samples were collected in plastic test tubes containing heparin and theophylline. Cyclic AMP was determined by the protein binding method of Brown *et al.*<sup>19</sup> as modified for plasma by Lattner and Prudhoe<sup>20</sup>. Bars indicate s.e.m. All subjects were physically healthy consenting adults. The lithium subjects (6 males, 3 females, ●) had a mean age of  $38 \pm 3.6$ . Mean plasma lithium level<sup>21</sup> was  $0.71 \text{ mEq l}^{-1}$  with a range  $0.32\text{--}1.15 \text{ mEq l}^{-1}$ . The no-lithium control subjects (7 males, 1 female, ○) had a mean age of  $34 \pm 3.6$ . The lithium subjects included four manic-depressive patients and one unipolar depressive patient, all in euthymic states; a hypomanic manic-depressive patient; an euthymic schizo-affective patient; and two normal individuals. The no-lithium subjects included one euthymic manic-depressive patient; one depressed unipolar patient; one agitated schizo-affective patient; one hypomanic manic-depressive patient and five normal individuals. Comparison (not shown) of the response of normal individuals without lithium to the response of patients without lithium does not suggest any effect of diagnosis or clinical state on the plasma cyclic AMP response to adrenaline. \* $P < 0.05$ ; \*\* $P < 0.01$ .

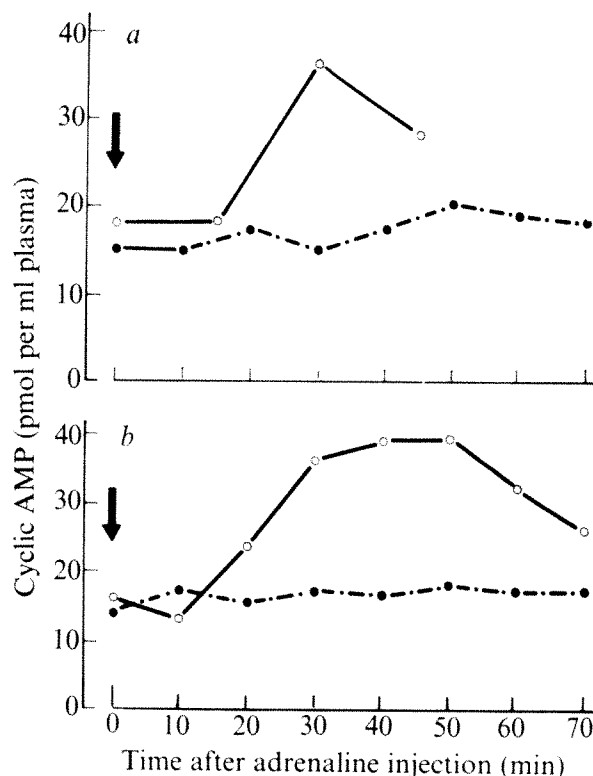
healthy volunteers leads to a rise in plasma cyclic AMP. Plasma cyclic AMP is derived from intracellular cyclic AMP of several tissues including liver, muscle and adipose tissue, but the rise in plasma cyclic AMP after  $\beta$ -adrenergic stimulation seems to result largely from stimulation of  $\beta$  receptors other than those in the liver or adipose tissue<sup>13</sup>. We have measured the rise in plasma cyclic AMP induced by adrenaline in subjects on and off Li therapy, and have found that therapeutic doses of the drug block this effect.

The effect of adrenaline on plasma cyclic AMP levels for a group of Li-treated and control subjects is shown in Fig. 1. In the control subjects there was a mean rise in plasma cyclic AMP levels which commenced at 10 min after adrenaline injection, peaked at 40 min, and by 70 min had almost returned to base levels. Li-treated subjects showed no mean increase in plasma cyclic AMP levels after adrenaline administration. Examination of individual responses (not shown) reveals that each of the no-lithium subjects showed a rise in plasma cyclic AMP levels after adrenaline injection, the magnitude of the maximum response ranging from 38 to 221%. In contrast, five of nine Li-treated subjects showed no rise in plasma cyclic AMP levels and the remaining four only a small increase (29–

39%). It should be noted that there seems to be no effect of Li on basal adenylate cyclase activity, but only on the activation of this enzyme by the hormone. Similar results have been reported for the ADH-sensitive adenylate cyclase in the kidney<sup>4</sup>. Figure 2 shows that two normal individuals (R.E. and H.B.) who respond to adrenaline with a rise in plasma cyclic AMP levels ceased to show a response when the test was repeated after 3 d of Li treatment. This suggests that the effect of Li on adrenaline-stimulated adenylate cyclase is a pharmacological effect of the drug and is not dependent on pre-existing pathology.

In addition to a direct effect of Li on adenylate cyclase, it has been proposed that this drug may also act at a site more distal to the synthesis of cyclic AMP<sup>14,15</sup>. Our results, however, suggest that a more distal action of Li need not be postulated to explain the effect of Li on the adrenergic system. Li's marked effect on  $\beta$ -adrenergic adenylate cyclase contrasts with the paucity of reports of Li effects on the peripheral cardiovascular system<sup>16</sup>. Intracellular levels of cyclic AMP after receptor stimulation are probably many times greater than those occurring in extracellular fluid<sup>12</sup>. It is possible that intracellular cyclic AMP responses, even after Li administration, are adequate to maintain homeostatic integrity. Fann *et al.*<sup>17</sup> have shown, however, that Li does decrease the pressor response to infused noradrenaline in man by 22%.

The catecholamine hypothesis of affective disorders postulates that mania is characterised by a functional excess of noradrenaline at a site in the central nervous system<sup>18</sup>. We have shown that in man, therapeutic doses of Li inhibit the peripheral  $\beta$ -adrenergic-stimulated adenylate cyclase. The brain adenylate cyclase stimulated by noradrenaline and the peripheral  $\beta$ -adrenergic-stimulated adenylate cyclase may be related, since both synthesise cyclic AMP after



**Fig. 2** The response of plasma cyclic AMP to adrenaline before (○) and after (●) Li in two normal subjects. After initially testing the response of plasma cyclic AMP to adrenaline in the absence of Li, both subjects were given Li for 3 d until a blood level of  $0.60 \text{ mEq l}^{-1}$  (R.E.) and  $0.66 \text{ mEq l}^{-1}$  (H.B.) was attained. The test was then repeated. The experimental procedure is the same as described in the legend to Fig. 1. a, Patient R.E.; b, patient H.B.

hormonal stimulation. This is in contrast to stimulation of the peripheral  $\alpha$ -adrenergic receptors, by either adrenaline or noradrenaline, which leads to a rise in cyclic GMP levels in plasma<sup>12</sup>. If Li inhibits human brain adenylate cyclase stimulated by noradrenaline, its antimanic efficacy could be understood.

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## Antagonism between channels for pattern and movement in human vision

MACKEY<sup>1</sup> rediscovered a striking experimentally produced visual hallucination. After inspection of stationary black-and-white stripes, a uniform test field is apparently filled with particles in chaotic streaming motion at right angles to the orientation of the stripes<sup>2</sup>. A great deal has since been discovered about pattern-analysing channels in the human brain, and much of this information has been gained from the study of visual illusions and after-effects. The streaming hallucination, however, has never been properly explained<sup>3</sup>, and is now not much more than a curiosity in the textbooks. In this paper an explicit model of the streaming after-effect is proposed, and from it some new subjective after-effects are derived.

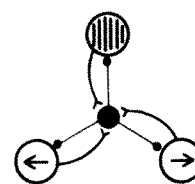
Physiological<sup>4</sup> and psychophysical<sup>5</sup> results imply that the visual system is organised into parallel subsystems of 'sustained' and 'transient' channels which have complementary roles in visual processing. Sustained channels code information about spatial form, pattern and contour, whereas transient channels primarily carry information about temporal change, flicker and movement. Sustained channels have higher spatial acuity, and selectivity<sup>4</sup>, whereas transient channels have higher temporal acuity, and in man are probably always direction selective<sup>6</sup>.

In the visual cortex, cells tuned for a particular contour orientation are grouped together in slab-shaped columns, along with cells whose 'preferred' direction of movement is

at right angles to that orientation<sup>7</sup>. Neighbouring columns have adjacent preferred orientations, producing a complete array of orientation columns<sup>8</sup>. We also know that mutual inhibition between neurones in a particular neighbourhood is a prime feature of cortical organisation<sup>9</sup>.

Suppose that, within a column, sustained (pattern) channels selectively inhibit movement channels and vice versa, as shown schematically in Fig. 1. It has frequently been suggested that to explain the motion after-effect ('waterfall illusion') opponent pairs of movement analysers are required<sup>10-12</sup>, so that in one sense the triplet model of Fig. 1 may be seen as an outgrowth of that idea. If the output of one channel is reduced by adaptation, then the resting level of the other two channels must rise since they are released from inhibition. Therefore, after adaptation to stationary vertical stripes there is a temporary increase in the outputs of the horizontal movement channels and horizontal streaming movements are seen. After inspection of moving stripes one of the movement channels is also adapted; only the opposite movement channel is released from inhibition, and streaming is seen in that direction only<sup>2</sup>.

Pattern channel



Movement channels

**Fig. 1** Model of antagonism between cortical channels extracting components of pattern and movement in retinal image. There is mutual inhibition between a pattern channel selective for vertical contours and movement channels sensitive to left or right movements. The three channels illustrated can be expected to lie within a single cortical orientation column. Other orientations would be handled by similar triplets in neighbouring columns. A single inhibitory interneurone is shown (black disk) but other ways of producing the interaction are possible (for example, an interneurone for each pair of channels) without altering the basic idea. The model can explain the streaming after-effect<sup>1-2</sup>, and predict some new phenomena (see text).

From the symmetry of the model two predictions can be made. Adapting to left and right movements without contours should release the corresponding pattern channel. Hallucinated stationary stripes at right angles to the movement should be seen as an after-effect. If adaptation was to only one direction of movement, then the hallucinated stripes should appear to drift in the opposite (unadapted) direction.

These predictions were tested on human observers by adapting to rotating dot patterns without coherent contours. Such a procedure should produce adaptation of movement channels with little corresponding pattern adaptation. Since the motion was circular, the predicted effect must be one of radial lines, at right angles to the adapting motion.

Two coarsely textured disks were seen superimposed by a half-silvered mirror close to the observer's eyes. Both disks rotated at 66 r.p.m. either in the same direction (clockwise) or in opposite directions. These conditions will be called single and double rotation respectively. After fixating the centre of this display for 1 min, the subject then gazed at the centre of a sheet of smooth white paper to observe any after-effects. His descriptions were recorded on tape and he also made drawings wherever possible. Each subject made two observations in each condition of rotation, and

these conditions were presented alternately. Half the subjects began with single rotation and half with double rotation.

After inspection of irregularly patterned disks rotating in the same or opposite directions most subjects spontaneously reported seeing complex patterns of radial lines or curves. There were also reports of fields of moving dots, or of no pattern at all. The taped reports were therefore analysed according to a fivefold classification: (1) stationary radial pattern, (2) moving radial, (3) stationary granular, (4) moving granular, (5) no pattern. After double rotation most reports were of stationary radial contours (50%) or of no pattern at all (Fig. 2). Two subjects reported patterns with some anticlockwise movement, but this was almost certainly a residual effect from the preceding trial of single rotation.

After single rotation half the reports were again of radial contours, but rotating anticlockwise, in the opposite

direction to the adapting motion. All but one of the remaining reports were of a field of dots in anticlockwise motion. In 80% of cases, the two observations in each pair fell into the same category of report; in the remaining cases, the first report was of no pattern or of moving dots, but the second report was of radial contours.

These results strongly support the proposed model of a mutual antagonism between three types of channel contained in each individual orientation column of the visual cortex. Adapting out one or two channels produces an 'hallucinated' after-effect based on the disinhibited or rebound response(s) of the remaining channel(s). Adapting to movement produces orthogonal contours (and vice versa, as MacKay's work<sup>1</sup> showed).

That a rotating field of dots (rather than contours) could be evoked suggests that sometimes only one of a disinhibited pair of channels may actually exceed the perceptual threshold. In this case it seems that the movement channel exceeded threshold but the pattern channel did not. As in the 'streaming after-effect' this seems to be characterised by the perception of moving granules. This interpretation is also consistent with the fact that some subjects continued to see movement for some time after the contours had faded.

Usually the after-effect would last only a few seconds, but during that time it could appear remarkably clear and vivid. Some of the subjects' drawings are reproduced in Fig. 2. Like subject 10, I see the lines as straight after double rotation but curved after single rotation. This difference was reported by several other subjects, but quite often curved lines were described even after double rotation. Following single rotation the apparent rotation of the hallucinated lines is about an order of magnitude faster than the conventional motion after-effect. In all cases the lines are confined to the adapted region of the visual field, and follow Emmert's law of apparent size when 'projected' on to a uniform field at different distances.

I have experimented less formally with dichoptic adapting conditions. Using a Dove prism, it was easy to adapt one eye to clockwise rotation while the other eye adapted to anticlockwise. Testing each eye separately I saw a pattern of radial curves rotating in a direction opposite to the adapting direction seen by that eye. That is, the adaptation seemed to be eye specific. Viewing the test field binocularly, however, I saw a stationary pattern of straight radial lines, just as obtained with binocular adaptation to double rotation. These observations were confirmed many times. Furthermore, as with the streaming after-effect, these contoured hallucinations did not transfer between the eyes, if one eye was adapted and the other was tested.

These observations with dichoptic adaptation can be understood if channels in the human brain have varying degrees of ocular dominance, as found for cells in cat and monkey cortex<sup>2</sup>. Testing the after-effect with one eye revealed the activity of monocularly dominated channels adapted to only one direction and thus adaptation seemed to be eye specific. If the tested eye had not been adapted, then no effect was seen, again implying eye-specific adaptation. Binocular testing did not give the sum or mixture of the two monocular percepts, but instead revealed the activity of binocular channels adapted to both directions. Thus the after-effect was stationary just as with ordinary binocular adaptation.

The subjective patterns and streaming movements generated by adaptation are therefore anything but a textbook curiosity. We can apparently use them to probe the columnar organisation and within-column interactions in the human visual cortex. There is considerable evidence for a more widespread inhibitory interaction between columns in the cortex, and this too shows up in the 'hallucinated' after-effects. Adapting to a sinusoidal grating gives rise to subjective patterns of other orientations and

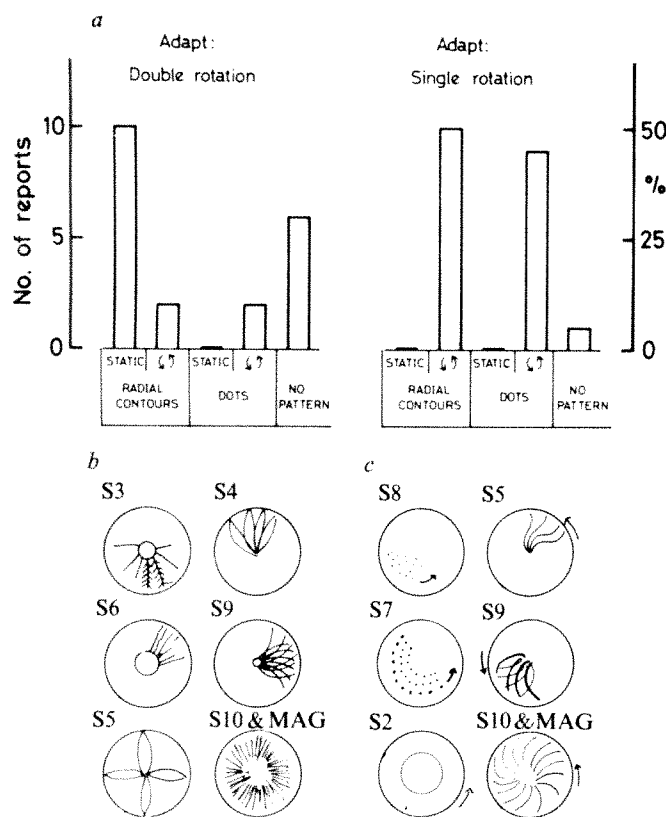


Fig. 2 *a*, Reports obtained from 10 naive observers after inspection of two superimposed dot patterns rotating clockwise (single rotation) or in opposite directions (double rotation) at 66 r.p.m. Disks subtended 12° at a viewing distance of 109 cm. Each disk bore a pattern of roughly circular dark spots on a white background. Each spot was about 0.5° across, drawn by hand in a quasi-random distribution, and separated by 0.5–1.5°. The contrast of each spot was about 0.4, relative to the combined background luminance ( $L_{max}$ ) of 26 cd m<sup>-2</sup>. (Contrast in this situation is defined as  $(L_{max} - L_{min})/L_{max}$ , where  $L_{min}$  is the luminance of the spot.) Patterns of smaller dots, or random visual noise were found to produce similar effects. *b* and *c*, Representative sample of subjects' sketches (redrawn). Where the lines fill only one quadrant of the drawing the observer invariably indicated that the whole region was filled with the same pattern. *b*, Adapting to double rotation produced stationary radial contours as an after-effect. *c*, Adapting to clockwise rotation produced radial contours (or a field of dots) rotating anti-clockwise. Appearance of curved lines (or cross hatching) as well as straight radial lines suggests that several near-radial orientations may be simultaneously disinhibited. The fact that the adapting speed increased with radial position on the disk might also have influenced the forms observed.

spatial frequencies<sup>13</sup>. These phenomena will be discussed in detail in a later paper.

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## Effects of ethanol injections on morphine consumption in morphine-preferring rats

If there is a connection between the systems influencing morphine and ethanol consumption as suggested by the results of refs 1–4, then injections of ethanol should affect the intake of morphine. We have tried to determine if alcohol would cause a depression in morphine consumption in morphine-preferring rats similar to the results reported with ethanol consumption and morphine injections<sup>5</sup>. The results show no such connection to exist.

The subjects were 17 male Hooded rats (Canadian Breeding Farms) weighing between 250–325 g at the start of the experiment. All rats were housed individually in stainless steel cages. Purina Lab Chow was available *ad libitum*.

In the first phase of the experiment, a preference for morphine solutions over tap water was established. Since oral intake of morphine is limited by the reluctance of animals to ingest opiates presumably because of their aversive taste<sup>6,7</sup>, we developed two procedures where non-nutritive saccharin was used to mask the taste of morphine. Eight rats were provided with a free choice between water and a morphine solution (0.1 g ml<sup>-1</sup>) adulterated with 0.5 mg ml<sup>-1</sup> sodium saccharin, both of which were continuously available. The criterion used for morphine preference was arbitrarily set at persistent consumption of 60% or more of the total fluid intake as morphine. If this level was achieved, the morphine concentration was increased to 0.2 g ml<sup>-1</sup>, whereas the saccharin concentration remained constant. If the rat continued to drink the morphine solution, its concentration was increased in steps of 0.1 g ml<sup>-1</sup> until a cutoff concentration of 0.5 g ml<sup>-1</sup> was attained. The 5 rats which developed a morphine preference comprised group 1. An additional 10 rats were provided with a free choice between tap water and a morphine solution (0.5 g ml<sup>-1</sup>) adulterated with 0.1 g ml<sup>-1</sup> of sodium saccharin. If the rats failed to drink 60% or more of their total fluid intake from the morphine/saccharin tube, the saccharin concentration was increased in steps of 0.1 g ml<sup>-1</sup> until the criterion for preference was attained, or until the arbitrary cutoff concentration of 0.65 g ml<sup>-1</sup> of saccharin was reached. The morphine concentration was kept constant throughout. Four animals developed a preference for the morphine/saccharin solution and subsequently made up group 2. Group 3 (*N*=8), a control group, was given a free choice between tap water and a 0.5 g ml<sup>-1</sup> solution of sodium saccharin.

Seven days after group 1 and group 2 developed a preference for the saccharin-adulterated morphine at their

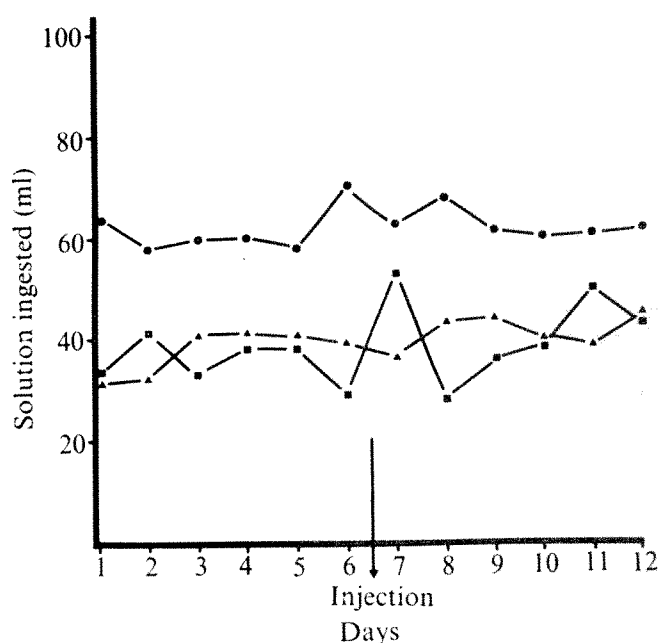


Fig. 1 Effect of ethanol injections on morphine intake over days. ●, Group 1; ▲, group 2; ■, group 3. The arrow represents injection of ethanol.

respective cutoff concentrations, the rats were injected intraperitoneally with 4 ml kg<sup>-1</sup> of 20% ethanol. Group 3 was given intraperitoneal injections of 20% ethanol (4 ml kg<sup>-1</sup>) after having consumed 60% or more of their fluid intake from the saccharin tube for seven days. Fluid intake was recorded for six days following the injection for all groups. Food intake for the groups was recorded seven days preceding injection of ethanol and for a 6-d postinjection period. Fluid and food intake were monitored daily the same time throughout the experiment.

Seven days preceding injection of ethanol group 1 drank a mean of 60.38 ml of saccharin-adulterated morphine. The mean consumption of the saccharin-adulterated morphine for 6 d after the injection was 62.76 ml. A Wilcoxon-signed ranks pairs test indicated that there was no significant difference in morphine/saccharin intake between the preinjection and postinjection periods (*T*=3, *P*>0.05). Similarly, food and water were not affected by the injection (*T*=5.6, *P*>0.05; *T*=5, *P*>0.05, respectively). In the 7-d preinjection period, group 2 drank from the morphine/saccharin tube a mean of 33.95 ml. The mean saccharin-adulterated morphine consumption for the 6-d postinjection period was 41.0 ml. Again, there were no significant differences in morphine/saccharin consumption between pre- and postinjection periods (*T*=3, *P*>0.05), and food and water intake were not affected by the ethanol injection (*T*=0.8, *P*>0.05; *T*=2.0, *P*>0.55 respectively).

Control group 3 drank a mean of 37.25 ml from the saccharin tube in the 7-d preinjection period although their mean saccharin consumption in the 6-d period after the injection was 41.3 ml. Ethanol did not affect their saccharin consumption (*T*=10, *P*>0.05), food consumption (*T*=5.6, *P*>0.05) or water consumption (*T*=17, *P*>0.05) (see Fig. 1).

The results of this study suggest that ethanol does not influence the oral consumption of saccharin-adulterated morphine. The assumption that a common connection exists between the systems influencing the consumption of morphine and alcohol does not seem to be supported by these results. Perhaps if the rats were exposed to the morphine solutions for longer periods of time, the alcohol effect would become manifest. In the present study rats were exposed to morphine solutions for about 40 d; Sinclair's subjects were exposed to alcohol for 141 d. Length of



exposure to a drug could conceivably be an important factor to consider in studies of this nature, and may explain the dichotomy between these findings and those of Sinclair. Furthermore, it is possible that a such commonality does exist, but that it is not a simple symmetric relationship.

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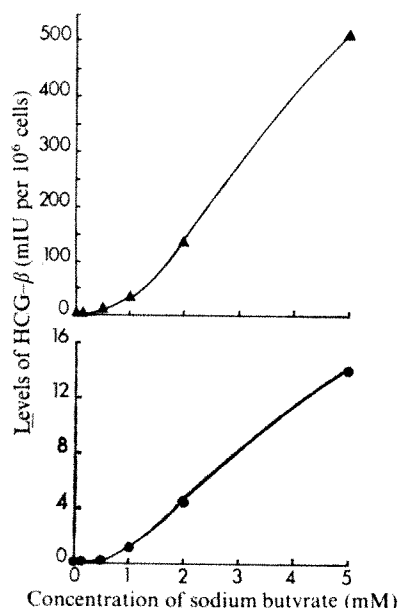
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## Production of human chorionic gonadotropin in HeLa cell cultures

THE ectopic synthesis of placental proteins in human cancer is of special interest because of similarities between neoplastic and embryonic cells. Moreover, the inappropriate synthesis of biologically active proteins and polypeptide hormones which are normally produced only in specialised tissues (endocrine glands) or at a particular stage of development may provide information on the control of gene activity. Ectopic production of human placental chorionic gonadotropin (HCG) has been reported in some patients with neoplasms<sup>1,2</sup>. A cell line (Cha Go) established from an HCG-producing pulmonary carcinoma was shown to continue synthesis of the gonadotropin in culture<sup>3,4</sup>. We have now found that HeLa<sub>65</sub> cells<sup>5,7</sup> produce the  $\beta$  subunit of HCG as measured by a specific radioimmunoassay and that sodium butyrate enhances production.



**Fig. 1** Production of HCG- $\beta$  by HeLa<sub>65</sub> cells grown for 120 h in the presence of different concentrations of sodium butyrate. ●, Intracellular HCG- $\beta$  (mIU per  $10^6$  cells); ▲, HCG- $\beta$  (mIU) secreted in 100 ml medium per  $10^6$  cells. HCG- $\beta$  was determined by radioimmunoassay using polyethylene glycol as a precipitant for <sup>125</sup>I-labelled antigen-antibody complex. Radioactive complexes were measured as outlined in Table 1 by counting in a Nuclear-Chicago Gamma-Counter.

**Table 1** Production of HCG- $\beta$  by HeLa<sub>65</sub> cells

Addition to medium	Intracellular HCG- $\beta$ (mIU per $10^6$ cells)		HCG- $\beta$ secreted in medium (mIU per $10^6$ cell)	
	Mean	Range	Mean	Range
None	0.07	0.06-0.08	0.06	0.059-0.061
1 mM sodium butyrate	2.18	2.15-2.21	62.3	59.3-65.3
3 $\mu$ M prednisolone	0.14	0.136-0.144	0.10	0.099-0.101

Replicate flasks of HeLa<sub>65</sub> cells were grown for 120 h in Waymouth's medium containing 10% foetal calf serum and antibiotics (penicillin, 50 U, streptomycin 50  $\mu$ g and kanamycin 30  $\mu$ g ml<sup>-1</sup>). The medium was decanted, freed from suspended cells by centrifugation and stored at -20 °C until assayed. The cells were removed from the glass surfaces with 0.02% EDTA and 0.05% trypsin (1:300 Grand Island Biological) in Puck's saline A. The cells were counted in a haemocytometer, washed by centrifugation in 0.15 M saline and frozen at -20 °C. Before assay the thawed cell pellet was suspended in 0.15 M saline and disrupted by sonification using Model W 185 Sonifier Cell Disrupter (Heat System—Ultrasonics).  $\beta$  subunits of human chorionic gonadotropin (HCG- $\beta$ ) were measured by a specific radioimmunoassay that exhibits less than 1% cross reaction with luteinising hormone (LH). Antiserum against the beta chain of HCG was used to ensure the specificity of the immunological reaction. Radioactive <sup>125</sup>I-HCG- $\beta$  antigen-antibody complexes<sup>16</sup> were precipitated with either anti-gammaglobulin serum or polyethylene glycol (molecular weight 6,000). The radioactive precipitate was collected by centrifugation at 4,000g in a refrigerated (4 °C) centrifuge and counted in a Picker Nuclear Autowell II Gamma-Counter. The concentration of HCG- $\beta$  in ultrasonicates of washed HeLa cells and in medium were calculated from a standard calibration curve. The standard curve was obtained by plotting %<sup>125</sup>I-HCG- $\beta$  bound to HCG- $\beta$  antibody against known concentrations of unlabelled highly purified HCG- $\beta$ . Standards were run concurrently with unknowns in each experiment. Purified HCG- $\beta$  (molecular weight 22,000) and purified HCG- $\beta$  labelled with <sup>125</sup>I and antibody against HCG- $\beta$  were supplied by Serono Laboratories, and also by the Institute of Bio-endocrinology, Montreal. Values are the means and ranges of duplicate assays carried out on two replicate flasks.

HCG is a heteropolymer composed of two dissimilar subunits; an alpha subunit that is common to follicle-stimulating hormone, luteinising hormone, thyroid-stimulating hormone and HCG and a  $\beta$  subunit unique for each hormone that confers biological specificity<sup>8</sup>. Table 1 shows that HeLa<sub>65</sub> cells produce low levels of HCG- $\beta$  and secrete it into medium. Growth of cells in medium containing sodium butyrate markedly increases HCG- $\beta$  production both intracellularly and in medium (Table 1). Similar striking effects were observed in ten additional experiments. The amount of HCG- $\beta$  produced by HeLa<sub>65</sub> cells depends on the concentration of sodium butyrate in the medium (Fig. 1). Sodium butyrate (0.1-10 mM), when added to the assay mixture, does not interfere with the radioimmune measurement of HCG- $\beta$ . Another well characterised HeLa subline designated HeLa<sub>71</sub> (ref. 6) also produced HCG- $\beta$  which was increased by growth in medium with sodium butyrate. On the other hand, the cortisol analogue, prednisolone, increases HCG- $\beta$  only slightly. In four other experiments, prednisolone slightly increased the gonadotropin levels in one but was without significant effect in the other three. In baby hamster kidney BHK/C<sub>13</sub> cultures<sup>8</sup> and adult human lung fibroblasts gonadotropin could not be detected nor could HCG- $\beta$  be induced by growing these cells in medium with either sodium butyrate or prednisolone (data not shown).

It is interesting that the heteroploid HeLa cell line after many years in culture produces several proteins normally found only during embryonic development or synthesised by specialised cells. For example, these cells produce the placental form of alkaline phosphatase with electrophoretic characteristics similar to the isozymes of the heterozygous FS phenotype<sup>9</sup>. The addition of iron to culture media mediates the synthesis by HeLa cells of an apoferitin with properties different from the adult form of this protein<sup>10</sup>. HeLa cells are agglutinated by concanavalin A, a property shared by tumour and embryonic cells (our unpublished

results). The production of HCG- $\beta$  by HeLa cells, as measured by a specific radioimmunoassay, provides additional evidence for the putative derepression of an element of the genome with the synthesis of a protein normally produced only by the specialised trophoblastic cells.

Cortisol<sup>11,12</sup> and more recently sodium butyrate<sup>13-15</sup> have been reported to modulate the shape and to alter the activities of several enzymes in HeLa cells. Short chain fatty acids such as butyrate increase the activity of HeLa alkaline phosphatase<sup>14</sup> and sialyltransferase<sup>15</sup>. The accumulation of HCG- $\beta$  in HeLa cell cultures is enhanced markedly by growth in medium containing sodium butyrate. Since this peptide hormone is not degraded in cell cultures<sup>4</sup>, it seems that the synthesis of HCG is increased by butyrate.

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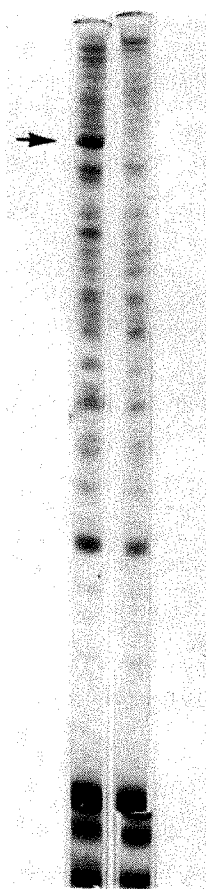
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## Loss of a non-histone chromatin protein parallels *in vitro* differentiation of cartilage

SEVERAL lines of evidence have implicated the proteins associated with the nuclear DNA of eukaryotic cells in the control of differential gene expression<sup>1-6</sup>. In contrast to the histones of eukaryotic chromatin which are relatively conserved in both primary structure and distribution across cell types within an organism, and from species to species<sup>4</sup>, the non-histone chromosomal proteins are very variable in size and distribution. This lends weight to the presumption that they are the specific regulators of transcription<sup>5,6</sup>.

Although there have been studies of tissue-specific<sup>5-8</sup>, developmental stage-specific<sup>9-11</sup> and cell-cycle phase-specific<sup>12-14</sup> variations in the complement of non-histone chromatin proteins, the causal association of specific alterations in chromosomal proteins with a particular transformation of one cell type into another can best be established with a homogeneous population of precursor cells able to differentiate in isolation into a homogeneous population of a recognisable cell type. In particular, differences in the chromosomal proteins of cell types which are adjacent in a developmental lineage would be more amenable to analysis than the larger number of differences likely to be encountered among cell types more widely diversified during development. Here we report a change in the complement of non-histone, chromosomal proteins in an *in vitro* system that closely approaches this situation.

Fig. 1 Gels prepared from chromatin proteins from nuclei of freshly explanted (left) and 3-d cultured (right) sub-apical ridge mesoderm from Hamilton-Hamburger stage 25 chick wing buds. Tissue was pooled from 370 White Leghorn embryos; nuclei were isolated from half the material immediately and half after 3 d in culture (see text). In each case intact pieces of tissue were suspended in a buffer consisting of 0.32 M sucrose, 0.001 M MgCl<sub>2</sub>, 0.3% Triton N-101 and 0.001 M phosphate buffer, pH 6.8. Tissue was homogenised with two strokes of a Teflon pestle in a Potter-Elvehjem homogeniser. Nuclei were collected at 1,000g and resuspended in 3 ml of the same buffer, washed in 80 mM NaCl, 20 mM EDTA, pH 7.2, and then in 0.15 M NaCl, 0.05 M Tris pH 7.5. The resulting pellet was dissolved in 2% sodium dodecyl sulphate (SDS) in 0.01 M Tris and 10% glycerol and boiled, after which 1 mM phenylmethylsulphonyl fluoride<sup>21,22</sup> was added. The mixture was then dialysed against 0.1% SDS, 0.01 M Tris, 0.3 M glycine, and run on Tris-glycine 10% polyacrylamide SDS gels using the gel and buffer systems of Laemmli<sup>23</sup>. Approximately 150  $\mu$ g of protein was applied to each gel. Gels were stained with Coomassie brilliant blue. The protein characteristic of the prechondrocyte preparation is indicated by the arrow.



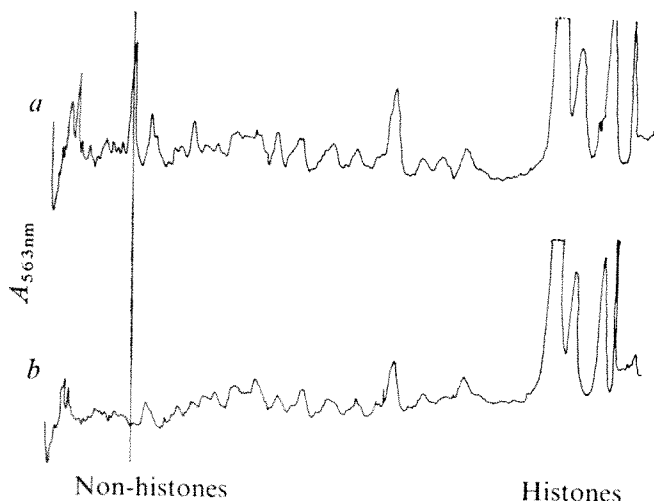
As described<sup>15</sup>, the mesenchyme subjacent to the apical ectodermal ridge<sup>15</sup> of the stage 25 (5-d) chick wing bud consists of cells which are not chondrocytes according to several criteria. The dissociated cells cannot clone for cartilage in conditions which support cartilage colony formation by authentic chondrocytes<sup>17</sup>; sectioned subridge mesoderm does not stain metachromatically with toluidine blue, a test which is diagnostic for the sulphated mucopolysaccharides of cartilage matrix<sup>18</sup>; and most definitively, the collagen produced by the subridge mesoderm is of a level (1.5-2.5% of total newly synthesised protein when grown in ascorbate), and species (type I-like) uncharacteristic of cartilage (ref. 19 and R. Mayne, unpublished results). Nonetheless, after 3 d in organ culture, explanted subridge mesoderm attains the cartilage phenotype by the following criteria: dissociated cells clone for cartilage; sectioned explants stain metachromatically with toluidine blue, and the level (14-16% of newly synthesised protein) and species (type II-like) of collagen indicate that the tissue has virtually completely chondrified. At the histological level the transition proceeds uniformly during the 3 d, with moderate DNA synthesis at all stages (determined autoradiographically) and no overt necrosis. No myotubes are ever seen in these explants, suggesting that while the freshly explanted material is not yet committed to a chondrogenic programme, it has nevertheless passed the stage in the mesodermal lineage when it can 'choose' to be myogenic<sup>20</sup>.

It is significant with regard to the possible mechanisms involved in this transition during development, that the individual  $\alpha$ -helical chains comprising chick type I collagen (found in skin, tendon, bone and various other tissues) differ in their primary structure from the  $\alpha$ -helical chains comprising chick type II collagen (found only in cartilage)<sup>19</sup>. The appearance of a new gene product in the subridge mesodermal cells or their descendants during

the 3d in culture suggested reprogramming at the chromosome level and led us to look for corresponding changes in the nuclear proteins of these cells.

Tissue was isolated from the sub-apical ridge region of 370 stage 25 chick wing buds by transection of the limb tip 0.2–0.3 mm from the apex. No attempt was made surgically to remove the ectoderm which we estimate contributed less than 10% of total cells to the newly isolated material. Also, as sheets of ectoderm were lost during the early steps of nuclear isolation, the contribution of ectodermal nuclei to the final chromatin protein sample was probably a few per cent. Nuclei were isolated immediately from half the tissue, and from these chromatin was prepared and stored in a denaturing buffer containing phenylmethylsulphonyl fluoride, an inhibitor of proteolysis<sup>21,22</sup>, at  $-20^{\circ}\text{C}$ .

The other half of the tissue was cultured on 1.2% agar containing Ham's F-10 medium supplemented with 10% foetal calf serum and 1% bovine serum albumin. After 3 d in culture, nuclei were isolated from this tissue by the same procedure, chromatin was prepared, and the two preparations were run simultaneously on 25 cm 10% polyacrylamide gels containing 0.1% sodium dodecyl sulphate. The stained gels and the corresponding visible light spectrophotometer scans in Figs 1 and 2 were made from the preparation described, in which the freshly explanted and cultured material was taken from the same group of randomised embryonic limbs. Replicate analyses were done from separately prepared fresh and cultured tissue, yielding gels virtually identical to those pictured.



**Fig. 2** Optical scans of the gels in Fig. 1 done at 563 nm on a Gilford spectrophotometer (a). Preparation from freshly explanted material. b, Preparation from cultured material. Tracing of prechondrocyte distinctive band is indicated by the line. The histone region may also contain some non-histone proteins.

In view of the phenotypic behaviour of the material analysed, we emphasise the following features of our electrophoretic data. First, we note the essential similarity between the two gels with respect to most of their stainable bands. While there are quantitative variations between some of the corresponding bands, this similarity appears greater than that usually found when different tissues or organs from a single individual or species are compared<sup>2-14</sup>. Second, the presence of a heavily staining band of low mobility (molecular weight between 125,000 and 150,000, estimated from markers in separate gels) in the freshly explanted, but not cultured material, is quite striking. This band is not present in similar gels of material prepared from cultured embryonic chick vertebral chondrocytes, fibroblasts or myogenic populations, though a low intensity band of similar mobility appears in chromatin preparations from primitive series chick erythrocytes (unpublished results of J.B., G.C.T.Y., and H. Holtzer). We do not exclude the possibility that the phenotypic differences between our freshly explanted and cultured material could be partly or entirely controlled by chromatin-associated molecules outside the limits of detection of our electrophoresis, staining, and scanning techniques. But we emphasise that our two preparations represented cell types which are adjacent in a developmental lineage and that the distinguishing protein (or class of proteins) was prominent against a relatively constant background, suggesting a role for this component in nuclear reprogramming. The all-or-none character of its disappearance (or loss of affinity for the stain) during the change from prechondrocyte to chondrocyte in culture recommends this system for *in vitro* studies of the mechanisms of cell differentiation.

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## Expression of parental histone genes in the intergeneric hybrid *Triticale hexaploide*

*Triticale hexaploide*, the intergeneric hybrid resulting from the cross of diploid rye (*Secale cereale*) and tetraploid wheat (*Triticum durum*), offers a unique opportunity for the study of the expression of genes which are derived from two dissimilar organisms but exist together in the same cells. I have investigated the expression of histone genes in triticale because the histones are an extensively studied class of proteins with individual components which range from being highly conservative in an evolutionary sense to being probably species specific. For example, the sequence of histone F2a1 from peas and cows differs in only two of 102 amino acids<sup>1</sup>, whereas different species of sea urchin have F1 histones of different electrophoretic mobilities<sup>2</sup>. I



have found that in wheat, rye and triticale the evolutionarily conservative histones seem to be identical. There are, however, differences in the F1 histones of wheat and rye, and the gene for one of the wheat F1 histones is not expressed in the hybrid triticale.

Histones were extracted from dark-grown coleoptiles of triticale and from the rye and wheat parents as previously described<sup>1</sup>, and were analysed electrophoretically on short and long one-dimensional gels by the method of Panyim and Chalkley<sup>4</sup> and on a new two-dimensional electrophoretic system.

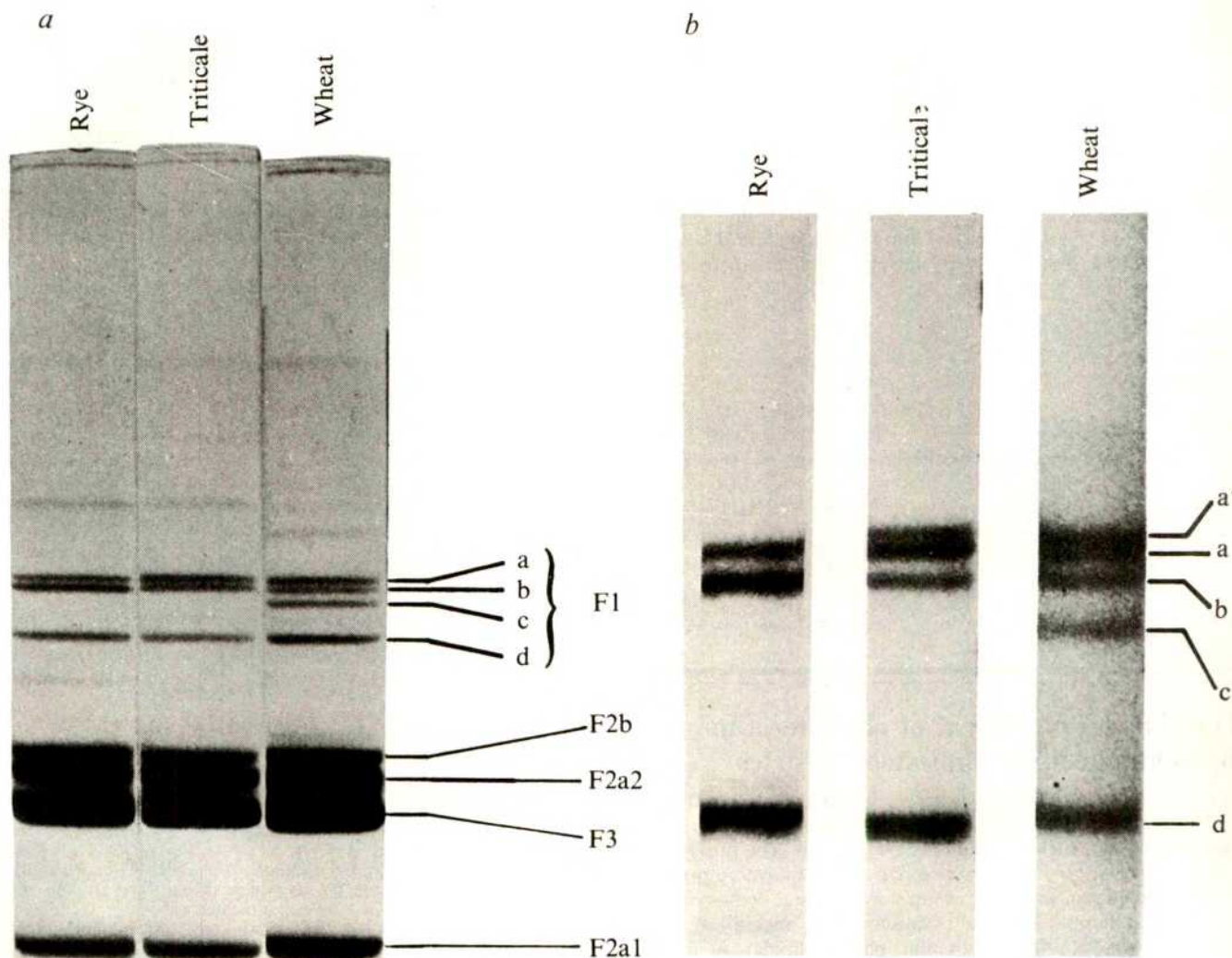
Not surprisingly, considering the relatively close phylogenetic positions of wheat and rye, the highly evolutionarily conservative histones (F2a1 and F3) and the moderately conservative histones (F2b and F2a2) from all three organisms appeared identical on both one and two-dimensional gels (Figs 1 and 2). The only differences were in the F1 (lysine-rich) histones.

On short gels wheat F1 histones were resolved into four distinct bands, F1a, b, c and d. Long gels further resolved

wheat F1a into two electrophoretic bands, the slower of which I have called F1a'. This heterogeneity of wheat F1 histone is not due to phosphorylation since treatment of the isolated F1 fraction with alkaline phosphatase by the procedure of Sherod *et al.*<sup>7</sup> did not affect the electrophoretic mobilities of the subfractions. Rye has only three F1 histones as resolved on both long and short gels. These have identical electrophoretic mobilities (as shown by electrophoresis of mixed samples of wheat and rye on long gels) to histones F1a, b and d (that is, rye histones are the same as wheat histones except that F1c and F1a' are lacking).

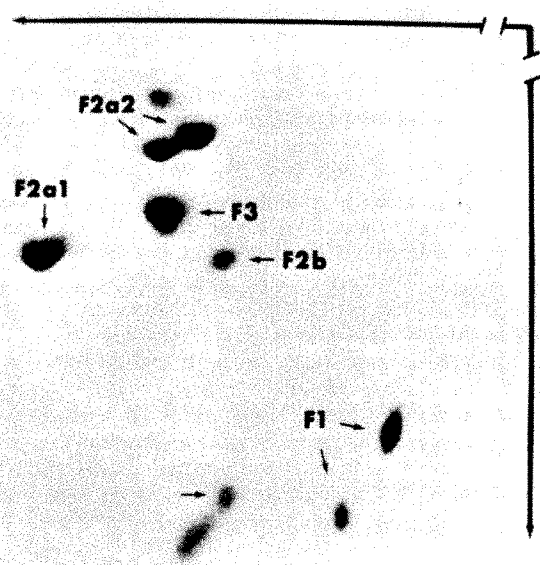
In the hybrid triticale the genes for histones F1a, b and d are expressed as is the gene for the wheat-specific F1a'. Even greatly overloaded gels, however, show no trace of the wheat-specific F1c histone.

The reason for the lack of expression of the wheat F1c gene is unknown, although it is not due to the loss of any whole chromosomes. Root tip squashes reveal the entire  $2n$  complement of 28 chromosomes derived from wheat and 14 derived from rye, in the hybrid.



**Fig. 1** *a*, Electrophoresis of wheat, rye and triticale whole histones on short gels by the method of Panyim and Chalkley<sup>4</sup>. The 10-cm gels were run for 4 h at room temperature, 2 mA per gel, then stained with amido black and destained by diffusion. All three species have histones F1a, b and d. The wheat-specific F1c is not expressed in the hybrid. The remaining histone fractions appear to be identical in all three plants. They have been named according to the nomenclature of Johns<sup>3</sup> and identified by their fractionation properties, susceptibility to ferric chloride destaining and comparative mobility in one- and two-dimensional electrophoresis (manuscript in preparation). In this electrophoretic system the plant F2b and F2a2 histones have lower electrophoretic mobilities than histone F3 whereas histones F2b and F2a2 of mammals have greater electrophoretic mobility than histone F3 (ref. 3). *b*, Electrophoresis of wheat, rye and triticale F1 histones on long gels. The gels are of the same composition as the short gels in *a*, but are 20 cm long and were run at 0.75 mA per gel for 50 h at 2 °C. Other histone fractions have run off the gel, and only the portion of the gel containing the F1 histones is shown. Rye histones appear the same as in short gels but are separated further. F1a has been resolved into two bands in wheat and triticale, the slower of which has been labelled F1a'. The wheat-specific F1c is not expressed in the hybrid.





**Fig. 2** Two-dimensional gel of triticate whole histone. Separation in the first dimension (right to left) was on acetic acid-urea gels according to Panyim and Chalkley<sup>4</sup>. The second dimension (top to bottom) was of the same composition but included 1% Triton X-100. The patterns for rye and wheat are identical except for the F1 histones. The rapidly moving, arrowed but unlabelled spots are probably histones which have not bound the Triton<sup>6</sup>.

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## Transbilayer movement of cholesterol in dipalmitoyllecithin-cholesterol vesicles

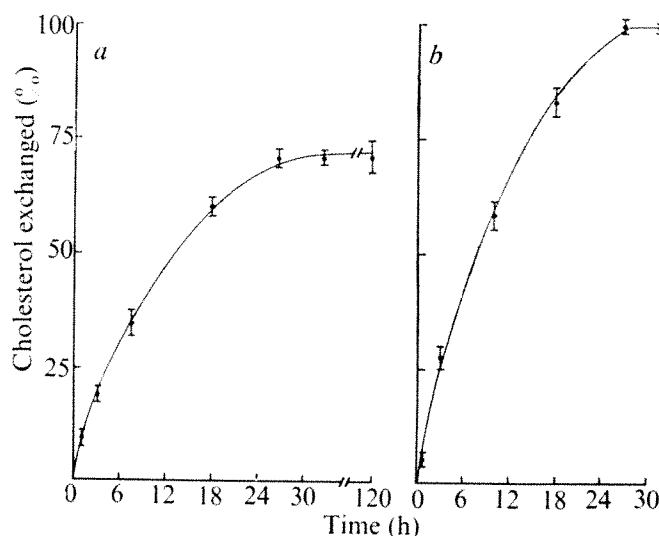
THE movement of cholesterol across biological membranes is an important process for many cellular functions, but the mechanisms by which it occurs are not known. The movement of lipid molecules between the two halves of a bilayer, a process which has come to be known as flip flop, was first demonstrated to occur in films of stearate<sup>1</sup>. Subsequently, several workers have reported flip-flop times for phospholipids in model systems, the values obtained varying between several hours and many days depending on the system studied and the techniques used<sup>2-5</sup>.

Lipid flip flop provides a possible mechanism for the translocation of cholesterol across membranes. To our knowledge only one study has been carried out to estimate the rate at which such a process could occur. Smith and Green<sup>6</sup> reported a half time of 70 min at 30 °C for the flip flop of the fluorescent cholesterol analogue, sterophenol, in liposomes. We have carried out experiments to measure the flip flop of cholesterol itself in sonicated dipalmitoyllecithin-cholesterol vesicles, a

well known system<sup>7</sup>. On the basis of two kinds of experiments using different strategies, we conclude that cholesterol flip flop in this system is a very slow process if it occurs at all.

Lipid vesicles were made by sonication of dipalmitoyllecithin (DPL) (Sigma) and cholesterol in the mol ratio of 1:0.9 at 4 °C for 1 h under nitrogen, followed by centrifugation at 20,000g for 45 min at 4 °C. The purity of the DPL was checked by thin-layer chromatography before and after sonication, and there was no evidence of impurities or chemical degradation. Red-cell ghosts were prepared using the technique of Dodge<sup>8</sup>. The ghosts were used in excess as an acceptor membrane and no attempt was made to measure their leakiness. Red cells were labelled with <sup>14</sup>C-cholesterol by incubating them in plasma containing <sup>14</sup>C-cholesterol<sup>9</sup>. Lipids were extracted from red cells<sup>8</sup> and from ghosts<sup>10</sup>, and cholesterol was determined by digitonin precipitation<sup>11</sup> and assayed by the method of Parekh and Jung<sup>12</sup>.

In the first set of experiments DPL-cholesterol vesicles containing <sup>3</sup>H-cholesterol and <sup>14</sup>C-DPL were incubated at a cholesterol concentration of 10<sup>-5</sup> M with red-cell ghosts at a cholesterol concentration of 4.2 × 10<sup>-4</sup> M. This represented a 42-fold excess of ghost membranes, so that back exchange of the tritiated cholesterol would be insignificant. The ghosts and vesicles were incubated at pH 7.4 in a phosphate buffer (1.2 mM MgCl<sub>2</sub>, 2.4 mM CaCl<sub>2</sub>, 4.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 1.7 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.0 mM KCl, 13.5 mM Na<sub>2</sub>CO<sub>3</sub>, 117.8 mM NaCl, 10.0 mM glucose) at 37 °C. At various times aliquots were taken, the ghosts separated from the vesicles by centrifugation, and both the supernatant and pellet assayed for <sup>3</sup>H and <sup>14</sup>C. To prevent sticking of the vesicles to the ghosts, 10<sup>-6</sup> M bovine serum albumin was added to the incubation medium. Figure 1a indicates that the exchange phenomenon saturates after 73% of the <sup>3</sup>H-cholesterol has been removed from the vesicles, in ~ 24 h. No additional cholesterol could be removed from the



**Fig. 1** *a*, Exchange of <sup>3</sup>H-cholesterol from DPL-<sup>3</sup>H-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the original sonicated mixture. % cholesterol exchange refers to the amount of vesicle cholesterol transferred to the acceptor membrane. Correction (never exceeding 7%) was made for vesicles sticking to ghosts at each time point. *b*, Exchange of <sup>3</sup>H-cholesterol from DPL-cholesterol vesicles into erythrocyte ghosts where the labelled cholesterol was introduced into the vesicles by a previous incubation with intact red cells containing <sup>3</sup>H-cholesterol. The vesicles were stored at 37 °C for 48 h before being added to the ghosts preparation. The incubation medium contained streptomycin sulphate as an antibacterial agent and 10<sup>-6</sup> M bovine serum albumin to reduce sticking of the vesicles to the ghosts. <sup>14</sup>C-DPL was used as a control to monitor the degree of sticking and the concentration of phospholipid in the supernatant containing vesicles. The disappearance of labelled cholesterol from the vesicles corresponds with the appearance of label in the ghost fraction.

Table 1 Ratio of  $^3\text{H}$  to  $^{14}\text{C}$  decays

Experiment	$\Delta t$ (h)	$R_1$ (ratio in vesicles)	$R_2$ (ratio in red cells)	$R_2/R_1$
1	0	120	80	0.67
2	0	69	48	0.69
3	0	68	51	0.75
1	20	120	87	0.72
2	20	69	52	0.75
4	20	240	163	0.68
2	43	69	50	0.72
Mean = $0.71 \pm .03$				

The third column gives this ratio ( $R_1$ ) for the vesicles at the end of the first incubation. The fourth column gives this ratio ( $R_2$ ) for the red cells at the end of a 2-h second incubation, which followed the first incubation after a delay  $\Delta t$ . See the text for experimental details. Ratios of  $^3\text{H}$  to  $^{14}\text{C}$  decays measured in red cell lipids and vesicles were determined in triplicate to within 5%. Error caused by the sticking of vesicles to red cells was determined separately and was found to be insignificant in our experimental conditions.

vesicles over a 120-h incubation period. Between 0 and 60 h the specific activity of the  $^3\text{H}$ -cholesterol in the vesicle decreases from  $14.9 \times 10^5$  d.p.m. per mg cholesterol to  $4.3 \times 10^5$  d.p.m. per mg cholesterol representing a 71% loss in labelled cholesterol. The specific activity of the ghost cholesterol increases from zero to  $0.3 \times 10^5$  d.p.m. per mg cholesterol, which is indicative of the large excess of cholesterol in the acceptor membrane. This 71% exchangeable and 29% non-exchangeable cholesterol pool in the vesicles correlates well with the evidence of Huang<sup>7</sup> that 70% of the total membrane cholesterol is located on the outer half of the vesicle bilayer, and suggests that the cholesterol on the inner half of the bilayer is not accessible for exchange. The possibility that the non-exchangeable cholesterol observed in these experiments indicated the presence of inner bilayers in the vesicles is excluded, since quantitatively similar results were obtained using sized vesicles that had been chromatographed on a Sepharose 4B column by the method of Huang, and are homogeneous by established criteria. A tentative conclusion is that cholesterol does not flip from the inner half of the bilayer to the outer half within the time of the experiment.

In a further experiment, 1:0.9 DPL-cholesterol vesicles containing  $^{14}\text{C}$ -lecithin but no  $^3\text{H}$ -cholesterol were incubated for 8 h with red blood cells containing  $^3\text{H}$ -cholesterol. The vesicles were then separated from the red cells and incubated with an excess of ghosts in the same manner as the previous experiment. Figure 1b shows that all of the  $^3\text{H}$ -cholesterol can be exchanged from the vesicles in 24 h. This is consistent with the conclusion that the cholesterol exchanged into the vesicles from the red cells did not flip to the inner surface and therefore remained on the outside available for exchange with the acceptor membrane.

In a different set of experiments designed to compare the size of the exchangeable pool with the total cholesterol pool, vesicles were made with DPL and  $^3\text{H}$ -cholesterol, and then  $^{14}\text{C}$ -cholesterol was exchanged into the vesicles by incubating them with red cells containing  $^{14}\text{C}$ -cholesterol for 4–5 h (first incubation). By introducing labelled cholesterol in these two ways, the  $^3\text{H}$ -cholesterol will be found throughout the vesicle, whereas the  $^{14}\text{C}$ -cholesterol will have been introduced only into regions of the vesicle reached by the exchange process. Following the first incubation the vesicles were isolated for a period  $\Delta t$ , after which the vesicles were incubated with unlabelled red cells to facilitate the exchange of cholesterol between the two systems (second incubation). The proportion of  $^3\text{H}$  and  $^{14}\text{C}$  present in the vesicles at the end of the first incubation and the proportion of the isotopes found in the red cells at the end of the second incubation were measured. The results of these experiments are given in Table 1. The ratio of  $^3\text{H}$  to  $^{14}\text{C}$  measured in the red cells was the same at the end of a second incubation of either one or two hours. Furthermore, this proportion was independent of  $\Delta t$  for values of this delay up to 43 h,

the longest time tested. It was found that the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  had a value in the red cells which was  $0.71 \pm 0.03$  of its value in the vesicles.

The finding that the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  in the red cells was different from that in the vesicles means that the exchange process does not sample vesicle cholesterol uniformly, or that all of the vesicle cholesterol is not available for exchange. The time independence of the results indicates that the pool of cholesterol molecules which exchange with red cell cholesterol constitutes an isolated subpopulation of the total cholesterol in the vesicle. If this exchangeable pool represents the outer layer of molecules, then the isolation of this pool from the rest of the vesicle cholesterol would imply that there is no translocation of cholesterol between the two surfaces of the vesicle.

Let us denote the number of  $^3\text{H}$  molecules in the exchangeable and non-exchangeable pools at the end of the first incubation by  $N_{H1}$  and  $N_{H2}$  respectively. All of the  $^{14}\text{C}$ -cholesterol in the vesicles will be in the exchangeable pool, and we denote their number by  $N_{C1}$ . The measured ratio of  $^3\text{H}$  to  $^{14}\text{C}$  decays in the vesicles at the end of the first incubation is:  $R_1 = (N_{H1} + N_{H2}) / N_{C1}$ . The cholesterol exchanged into the red cells during the second incubation came from the vesicle exchange pool and the ratio of  $^3\text{H}$  to  $^{14}\text{C}$  decays in the red cell is therefore:  $R_2 = N_{H1} / N_{C1}$ . Algebraic manipulation yields  $N_{H2} / N_{H1} = (R_1 - R_2) / R_2$  or  $N_{H1} / (N_{H1} + N_{H2}) = R_2 / R_1 = 0.71 \pm 0.03$ .

In these experiments, the number of  $^{14}\text{C}$ -cholesterol molecules in the vesicles was negligible compared with the number of  $^3\text{H}$ -cholesterol molecules and therefore the value of 0.71 gives the fraction of vesicle cholesterol molecules which are accessible for exchange. As remarked before, this number is in good agreement with published values<sup>7</sup> for the fraction of cholesterol molecules located at the outer surface of the DPL vesicle, and we conclude therefore that the exchangeable pool is the outer surface of the vesicle. That the exchangeable pool is isolated from the rest of the cholesterol in the vesicle is then the statement that cholesterol flip flop is extremely slow. Within the accuracy of our measurements, we can determine a lower bound for the half time to be 6 d. This lower bound was arrived at by taking values of 0.67 and 0.75 for the ratio  $R_2/R_1$  for time delays of 0 and 20 h respectively, to fit an exponential process. This gives an extremely conservative estimate for the half time of flip flop. Similar rates of flip flop have been observed for phosphatidylcholine<sup>3,4</sup>.

Slow cholesterol flip flop could have interesting implications for biological membranes. The slow rates observed for this process in vesicles may also occur in biological membranes which would explain the finding of some authors<sup>14</sup> that there exists a pool of erythrocyte membrane cholesterol which is not exchangeable.

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## Identification of $\beta$ carbolines isolated from fluorescent human lens proteins

WITH ageing, a number of significant changes occur in the chemistry of the human lens. In the central region of the organ there is a slow transformation of protein to high molecular weight (HMW) aggregates greater than  $50 \times 10^6$  (refs 1 and 2). It has been suggested that the relatively high concentration of such protein species in older lenses may cause significant light scattering<sup>3,4</sup> and may be a contributing factor in the development of senile cataract characterised by central sclerosis and opacification<sup>1</sup>. An age-dependent increase in the insoluble protein fraction has also been observed<sup>5-8</sup> and recent experiments indicate that the HMW species is an intermediate in the formation of this material<sup>15</sup>. Marked changes in the polypeptide chains of human lens proteins probably largely attributable to post-translational transformation, have also been observed<sup>16</sup>.

A yellowing of lens with age is also observed and its generation has been correlated with a concomitant increase in a protein bound non-tryptophan fluorescence<sup>8</sup>. This fluorescence is found primarily in the insoluble protein<sup>8,9</sup> fraction of the nuclear region as well as with the HMW components of the soluble protein fraction<sup>8</sup>. It also has been shown that this fluorescence is associated with a 43,000 molecular weight polypeptide found in a number of HMW species and the insoluble fraction<sup>8</sup>. This polypeptide seems to increase in concentration with age and is not present in very young lens.

Alkaline degradation of either the insoluble fraction or HMW fractions in 4 N barium hydroxide at 110 °C for 24 h results in the release of a number of fluorescent components which have been separated and partially purified by preparative paper electrophoresis at pH 1.9. The principle fluorescent compound obtained by this procedure was designated 'C<sub>1</sub>'. We report here the structure of this component and consider its relationship to the fluorescent material present in the lens proteins.

Approximately 1–2 g insoluble protein fraction obtained from 50–100 cataractous lenses were hydrolysed by 4 N barium hydroxide and C<sub>1</sub> was isolated by preparative paper electrophoresis at pH 1.9. Examination of C<sub>1</sub> by high pressure liquid chromatography using poragel PN in ethanol–water indicates that it contains considerable contamination and that only small amounts of it are present in the initial electrophoretically isolated preparations. C<sub>1</sub> has a yellow fluorescence when observed by long wavelength ultraviolet light (maximum 360 nm), is unstable and decomposes to two components characterised by a blue fluorescence designated Ia and Ib. These decomposition products have a much higher mobility towards the negative pole at pH 1.9 than their precursor C<sub>1</sub> and they are formed in all conditions including storage at –80 °C in the dry state. Oxidation of C<sub>1</sub> by potassium dichromate<sup>10</sup> yields only two components which are the same as Ia and Ib on the basis of electrophoresis and fluorescent colour. Thin-layer chromatography (silica gel–EtOAc) indicated that Ia (r.f. = 0.20) was present in much greater amounts than Ib (r.f. = 0.25). Oxidation of the barium hydroxide hydrolysate of the insoluble protein yields two components which are the same as the products obtained from oxidation of C<sub>1</sub> on the basis of electrophoresis, thin-layer chromatography and fluorescence. Much higher yields of Ia and Ib are obtained by this procedure than through isolation and oxidation of C<sub>1</sub>. This is undoubtedly attributable to the decomposition of C<sub>1</sub> which occurs during the hydrolysis and isolation procedures. A third fluorescent component was also observed. The origin, structure and significance of this third component are under investigation.

Studies on well characterised tryptophan-containing proteins such as bovine serum albumin failed to reveal the generation of any fluorescent components by alkaline hydrolysis<sup>10</sup> using the methods described above. Furthermore, with such methodology, essentially no fluorescent components were found with either

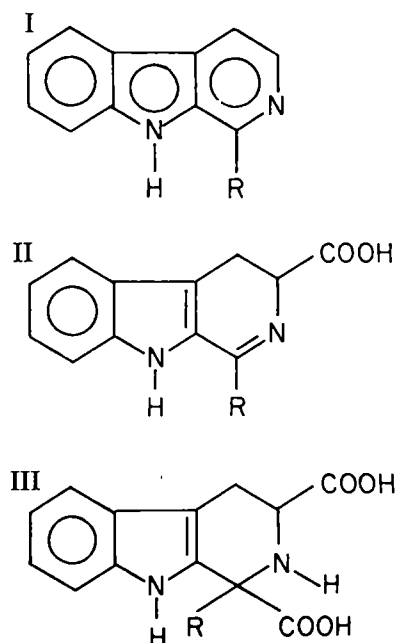
very young human lens proteins or calf lens protein. (Note that C<sub>1</sub> has been obtained by proteolytic digestion of succinylated, reduced and carboxymethylated protein from old human cataractous lenses<sup>8</sup>. Such protein is poorly digested and this approach is therefore not suitable for isolation and characterisation studies.) Hydrolysis of the above preparations in constant boiling hydrochloric acid<sup>11</sup>, however, revealed significant amounts of components comparable with C<sub>1</sub>. The amount of presumptive C<sub>1</sub> could be decreased but not eliminated by the presence of a reducing agent such as thioglycolic acid during the hydrolysis. Such observations suggest that C<sub>1</sub> was an alkaline hydrolysis product of precursors present in the insoluble lens protein and HMW fractions. In acid conditions, however, it was generated from normal tryptophan-containing proteins during the course of hydrolysis. The properties of C<sub>1</sub> and a consideration of the chemistry involved suggested that it might be a mixture of tryptophan derivatives, namely the 3,4-dihydro- $\beta$ -carboline-3-carboxylic acids IIa and IIb (Fig. 1) and that Ia and Ib were fully aromatic  $\beta$ -carbolines. Synthesis<sup>10,12,13</sup> of authentic Ia, Ib, IIa and IIb confirmed this hypothesis.

The following chromatographic and spectral data were obtained from both synthetic and natural Ia (about 15  $\mu$ g per 750 mg insoluble protein): thin-layer chromatography (silica gel–EtOAc), r.f. = 0.20; ultraviolet (in EtOH), 348, 335, 320s, 305, 287, 282, 267 and 250 nm; fluorescence (uncorrected, in EtOH), excitation at 350, 338, 290 and 257 nm, emission at 385 nm; mass spectrum, 182 (100%, M<sup>+</sup>), 181 (33%, M<sup>+</sup>–H), 154 (33%, M<sup>+</sup>–H–HCN), 127 (14%, M<sup>+</sup>–H–2HCN).

For synthetic and natural Ib (about 7  $\mu$ g for 750 mg insoluble protein) the following chromatographic and spectral data were obtained: thin-layer chromatography (silica gel–EtOAc), r.f. = 0.25; ultraviolet (in MeOH), 349, 335, 288, 282, 250s nm; fluorescence (uncorrected, in EtOH) excitation at 350, 338, 290 and 257 nm, emission at 385 nm; mass spectrum, 168 (100% M<sup>+</sup>), 167 (10%, M<sup>+</sup>–H), 140 (35%, M<sup>+</sup>–H–HCN), 114 (18%, M<sup>+</sup>–2HCN), 113 (14%, M<sup>+</sup>–H–2HCN).

As stated earlier C<sub>1</sub> is unstable and furthermore, since the amount of C<sub>1</sub> isolated from the lens is very small, direct spectral studies of this material were very difficult. Comparisons of electrophoretic and chromatographic data of C<sub>1</sub> with those of a mixture of authentic 1-methyl-3,4-dihydro- $\beta$ -carboline-3-carboxylic acid (IIa) and 3,4-dihydro- $\beta$ -carboline-3-carboxylic

Fig. 1  $\beta$ -carboline structures. I, Unsaturated  $\beta$ -carbolines; II, 3,4-dihydro- $\beta$ -carboline-3-carboxylic acid, III, 1,2,3,4-tetrahydro- $\beta$ -carboline-1,3-dicarboxylic acid. In the a form R = H; in the b form R = CH<sub>3</sub>.



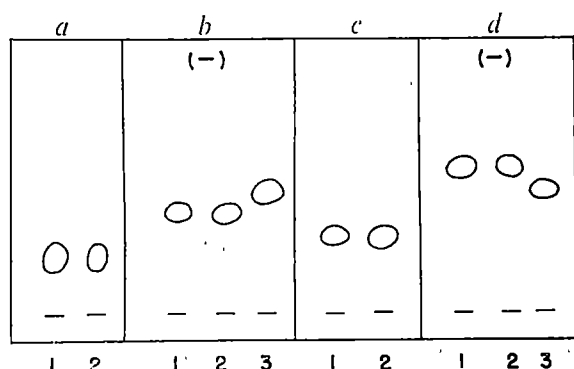


Fig. 2 *a*, Thin-layer chromatography comparison of (1)  $C_1$  and (2) a mixture of Ila and Iib (cellulose-0.01%  $NH_4OH$ ). *b*, Electrophoretic comparison of (1)  $C_1$ , (2) a mixture of Ila and Iib and (3) tryptophan (pH 1.9, 2,500 V, 25 min). *c*, Thin-layer chromatography comparison of methyl esters of (1)  $C_1$  and (2) Ila (silica gel-EtOAc). *d*, Electrophoretic comparison of (1)  $C_1$  methyl ester, (2) Ila methyl ester and (3) tryptophan (pH 1.9, 2,500 V, 25 min).

acid (Iib) demonstrated that they consisted of the same components (Fig. 2*a* and *b*). Note that the oxidation of  $C_1$  yields Ia and Ib, which are also obtained by oxidation of Ila and Iib, respectively. Ila and Iib on standing also decomposes to Ia and Ib with Iib being much less stable than Ila. Thus there is a disproportionate loss of Iib during the isolation of  $C_1$ , which consequently explains the small amounts of Ib obtained from the oxidation of  $C_1$ .  $C_1$  and Ila had the same fluorescent characteristics (excitation at 335 nm, emission at 495 nm; uncorrected in 0.01%  $NH_4OH$ ) and their methyl esters showed the same mobility on thin-layer chromatography and electrophoresis (Fig. 2*c* and *d*).

$C_1$  is probably a degradation product formed during the alkaline hydrolysis process. Therefore, three questions remain to be answered: Does the  $\beta$ -carboline ring structure exist *in vivo* in the lens protein? If so, in what oxidation state does it exist, and how is it linked to the lens proteins? We do not have direct evidence pertaining to the first question at this time, but the following experiment is suggestive. Protein from various sources were hydrolysed with base, oxidised with potassium dichromate, neutralised, extracted with ether and spotted on thin-layer chromatography. Protein obtained from 45- and 77-yr-old normal human lenses contained Ia and Ib. But significantly, essentially none of these components was detected in the protein of young human and calf lenses or bovine serum albumin. This suggests that there is an age-dependent formation of the  $\beta$ -carboline skeleton in human lenses. The second and third questions are more difficult to answer; however, preliminary experiments suggest that the *in vivo* precursor to the 3,4- $\beta$ -carboline-3-carboxylic acids Ila and Iib are 1-methyl-1,2,3,4-tetrahydro- $\beta$ -carboline-1,3-dicarboxylic acid (IIIa) and 1,2,3,4-tetrahydro- $\beta$ -carboline, 1,3-dicarboxylic acid (IIIb) (Fig. 1).

Base hydrolyses of synthetic IIIa (ref. 12) indeed gave the 3,4-dihydro- $\beta$ -carboline-3-carboxylic acid (IIa). These dicarboxylic acid structures are particularly attractive since they can act as a cross link between two polypeptide chains. Studies on the fluorescent 43,000 molecular weight polypeptide support this hypothesis. There is essentially no protein synthesis in the central region of the lens<sup>14</sup>. Thus the age-dependent appearance of the 43,000 molecular weight fluorescent polypeptide in this region must be attributable to the cross linking of two or more lower molecular weight components.

The mechanism by which the  $\beta$ -carbolines are formed in the lens is not understood at present. It is probable, however, that tryptophan is the precursor reacting with an unknown compound to form the  $\beta$ -carboline structure. This problem is now under investigation.

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## Light-induced fast conformational change in all-trans-retinal at low temperature

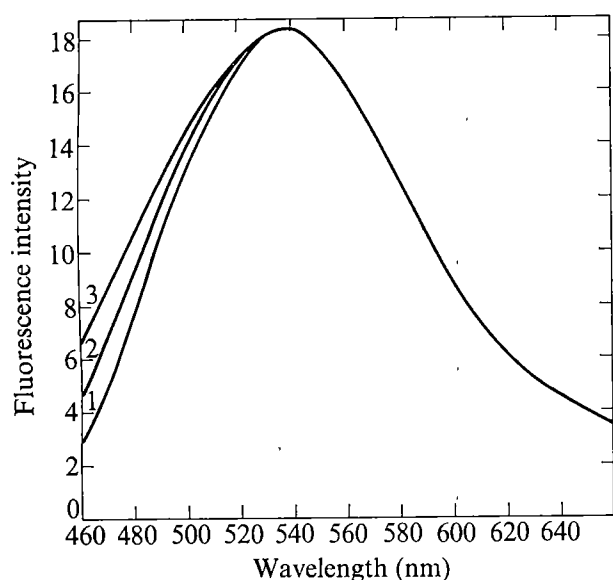
AN understanding of the excited state properties of retinal is essential for the elucidation of the mechanism of the visual process, because the photochemistry of the visual chromophore accounts to a large extent for the early bleaching stages of rhodospin<sup>1</sup>. All-trans-retinal has some unusual optical properties. It exhibits a dependence of its fluorescence quantum yield on the exciting wavelength<sup>2,3</sup> as well as an anomalous heavy atom effect<sup>4</sup>. In addition, the nature of its emitting state has not been determined; there is evidence<sup>5-7</sup>, however, for the presence of a low lying, weakly allowed <sup>1</sup>A<sub>g</sub> state in some other polyenes.

I report here the nanosecond fluorescence properties of all-trans-retinal in a non-polar matrix at 100 K. Two samples of all-trans-retinal (Sigma and Eastman Kodak) were used with identical results. The purity of the samples was ascertained by thin-layer chromatography<sup>4</sup>. A nanosecond fluorometer with a boxcar averager<sup>8,9</sup> was used. A 7-60 Corning filter was used for excitation; the fluorescence quantum yield is constant<sup>2</sup> when exciting in the spectral region in which the filter transmits (maximum transmission is at ~360 nm).

Nanosecond time-resolved fluorescence spectra in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K are shown in Fig. 1. A broadening of the spectrum with time in the nanosecond range is observed. The full width to half maximum (FWHM) of the spectrum obtained at 10 ns after the peak of the exciting light pulse is 127 nm; that of the spectrum obtained at 1.5 ns before the peak of the exciting light pulse is 113 nm. But the spectral maximum as well as the long wavelength portion of the spectrum are invariant with time.

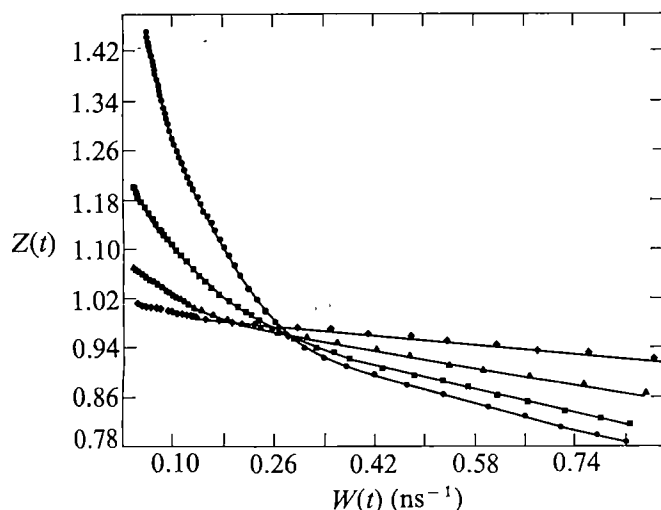
Figure 2 shows a strong dependence of the form of the decay on the emission wavelength. At 460 nm the decay is non-exponential; there is a long component corresponding to a decay time of about 3 ns at long times (small values of  $W(t)$ ) and a much shorter component corresponding to a decay time of about 0.3 ns at short times. As the emission wavelength increases, the decay becomes faster but it continues to be non-exponential up to about 510 nm. At





**Fig. 1** Normalised time-resolved fluorescence spectra of a  $10^{-6}$  M solution of all-*trans*-retinal in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K. The spectra were taken at the following times relative to the peak of the exciting light pulse (taken as zero time): (1) -1.5 ns; (2) +4 ns; (3) +10 ns. FWHM of the exciting light pulse  $\approx 4$  ns. A 7-60 Corning filter was used for excitation. The emission was analysed using a high intensity Bausch and Lomb scanning monochromator at a bandwidth of 19 nm. The spectra were not corrected for the variation of the photomultiplier-monochromator sensitivity with wavelength.

longer wavelengths the decay is virtually single exponential and independent of the emission wavelength; a decay time of about 0.1 ns is obtained in this wavelength region. It is significant that the variation of the form of the fluorescence decay with the emission wavelength occurs in the wavelength region for which the fluorescence spectrum is time dependent; this suggests that these two phenomena have a common origin. It should be noted that a decay time of



**Fig. 2** Phase-plane plots for the fluorescence decay of a  $10^{-6}$  M solution of all-*trans*-retinal in a mixture of 1:1 by volume of isopentane and methylcyclohexane at 100 K for the following emission wavelengths:  $\bullet$ , 460 nm;  $\blacksquare$ , 480 nm;  $\blacktriangle$ , 500 nm;  $\blacklozenge$ , 560 nm. The data were deconvoluted according to the method of Demas and Adamson<sup>12</sup>. Long times correspond to small values of  $W(t)$ . For a single exponential decay a phase-plane plot yields a straight line of negative slope equal to the decay time. A 7-60 Corning filter was used for excitation. The emission wavelengths were selected by using a high intensity Bausch and Lomb monochromator at a bandwidth of 19 nm.

$\sim 0.6$  ns has been reported<sup>4</sup> in absolute ethanol at 77 K; this value apparently refers to the long wavelength region.

Calculations<sup>10</sup> have shown that, as a result of electronic delocalisation brought about by excitation, the excited state-equilibrium torsional angle  $\phi_{6-7}$  between the cyclohexene ring and the polyene chain of all-*trans*-retinal is very different from that in the ground state. I infer then, that immediately after light absorption, relaxation will occur from the Franck-Condon electronic state, which is associated with the ground-state conformation, to an equilibrium excited state associated with a modified conformation. I therefore attribute the time and emission wavelength-dependent phenomena reported here to a relaxation process in the excited state resulting from rotation of the polyene chain about the C6-C7 bond. The relaxation time for this process depends of course on the microviscosity of the molecular environment. The spectral broadening observed in the nanosecond range implies that, under my experimental conditions, the relaxation process competes efficiently with the deactivation of the excited state by radiative and radiationless processes.

The results of a picosecond study<sup>11</sup> indicated that the formation of prelumirhodopsin when rhodopsin is irradiated probably involves a restricted change in the geometry of retinal rather than a complete isomerisation. The light-induced conformational change reported here may well be important in this first event of the visual process.

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## Erratum

In the article "Persistence of CMV genome in lymphoid cells after congenital infection" by J. H. Joncas, J. Menezes and E.-S. Huang (*Nature*, **258**, 432; 1975) the correct name of the third author should be Eng-Shang Huang. In line 7 of the article C. H. Huang should read E.-S. Huang.

## Nature Index and Binders

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# matters arising

## Algal sexuality

CAVALIER-SMITH<sup>1</sup> raises many interesting points on the origin of the eukaryotic cell and its organelles. I find myself in general agreement with him and with others<sup>2-4</sup> who have provided an alternative to the widely held theory that eukaryotic organelles were originally acquired by endosymbiosis. I wish to comment, however, on one aspect of his evolutionary scheme (see ref. 1, Fig. 5), namely, the assertion that sex is absent in the Dinophyceae, Euglenophyceae, and Cryptophyceae. In fact, there have been several reports<sup>5-7</sup> of sexuality in the Dinophyceae. Beam and Himes<sup>8</sup> have observed pairing between cells of *Cryptothecodinium cohnii* and their fusion into a single cell, followed by nuclear fusion. Furthermore, those workers<sup>8</sup> and others<sup>9</sup> have demonstrated genetic recombination in *C. cohnii*. Although there has been no such conclusive demonstration of sexuality in the Euglenophyceae, it is necessary to approach this negative evidence cautiously—especially since there is evidence of a meiotic process in two genera of this group<sup>10,11</sup>. One can only repeat Leedale's careful conclusion that "It seems probable that there is no sexuality in the majority of euglenoids, but it remains a possibility that the process does occur as a rare phenomenon; this can never be disproved". Finally, there is a dearth of information on the Cryptophyceae, and consequently it is premature to conclude that sexuality is absent in that group.

These observations do not detract from Cavalier-Smith's main arguments. If anything they strengthen his evolutionary scheme since, if sex does exist in the Dinophyceae, Euglenophyceae, and Cryptophyceae, one could move those groups from their isolated position on Fig. 5 of ref. 1 to a position among the other algae and speculate on their interrelationships. (For example, how are the Dinophyceae and Cryptophyceae related to other algae whose chloroplasts contain chlorophylls *a* and *c*?)

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## Irradiation and DNA breaks

JOHANSEN and Boye<sup>1</sup> reported their findings on the controversial question of whether the initial number of breaks induced by ionising radiation in DNA in oxic and anoxic conditions are identical, or whether less breaks occur in nitrogen than in oxygen. Their conclusion supports the latter.

They found<sup>1</sup> the difference in the yield of breaks in cellular DNA when *Escherichia coli* was irradiated in oxic and anoxic atmospheres and to minimise rapid repair<sup>2</sup> as far as possible, the time from irradiation to lysis of the cells was, according to those authors, "a fraction of a second". They assumed that cellular repair enzymes would not rejoin breaks in this short time and on the basis of some theoretical extrapolations on the correlation between the yield of breaks at a given dose and the rate of rejoining by cellular enzymes, they believed that the differences in yield of breaks due to rapid repair was an erroneous conclusion. They assumed that rejoining of breaks would not have occurred and if so, that the initial yield of breaks in oxic irradiation was at least three times more than that in anoxic conditions. I wish here to analyse the status of this controversy and I disagree with Johansen and Boye<sup>1</sup>.

The subject has long been a matter of debate and Dean *et al.*<sup>3</sup> were the first to suggest that anoxic breaks were subject to rapid enzymatic repair, showing that in experimental conditions which inhibited rejoining of strand breaks, nearly identical yields were obtained following oxic and anoxic irradiation.

The isolation of a DNA polymerase I mutant<sup>4</sup> and the study of the kinetics of single-strand break repair in *polA1* mutant<sup>2</sup> led to the recognition of a rapid repair system occurring in *E. coli*. The work on cellular DNA involved complications because repair probably

works very fast, rejoining breaks during irradiation and up to the moment of lysis. Town *et al.*<sup>5</sup> attempted to overcome the problem by preinactivating the cells at 52 °C for 10 min before irradiation and they obtained identical yields of breaks. Heat treatment, however, induces single-strand breaks in DNA<sup>6</sup> and release of sulphhydryl compounds in the medium<sup>1</sup>.

Another approach<sup>7</sup> was to irradiate bacteriophage  $\lambda$  extracellularly in oxic and anoxic atmospheres. Contrary to the situation for cellular DNA, any change in phage DNA would thus remain unmodified and beyond the influence of cellular repair as long as the DNA was not injected into a host. DNA could safely be extracted and layered on an alkaline sucrose gradient<sup>7</sup>. Identical yields of DNA breaks were obtained in oxic and anoxic extracellular irradiation<sup>7</sup>. When  $\lambda$  DNA was irradiated intracellularly in a *polA1* host, the decrease in molecular weight of  $\lambda$  DNA was identical in both oxic and anoxic conditions and equalled that obtained after extracellular irradiation. Intracellular anoxic irradiation of  $\lambda$  DNA to the same dose in wild-type bacteria, yielded no breaks<sup>7</sup>. This demonstrates that there is no difference in the initial number of breaks and that a rapid repair system, involving DNA polymerase I, rejoins breaks very rapidly. Similarly, there are data<sup>8,9</sup> on the equal number of breaks in oxic and anoxic conditions with phage T3 and T7 irradiated extracellularly.

Wild-type bacteria were injected for 10 min with  $\lambda$  DNA which had been irradiated extracellularly with 50 krad in oxic and anoxic conditions.  $\lambda$  DNA was immediately analysed for single-strand breaks.  $\lambda$  DNA irradiated anoxically had no breaks, but a few could be seen in DNA irradiated in oxygen. Since the initial yields of breaks were identical<sup>7</sup>, the difference in molecular weight after infection is attributable to cellular repair.

Sulphydryl compounds may have a function in the radiation sensitivity of *E. coli*<sup>1</sup> but I feel that the experiments described (ref. 7 and above), supported by other results<sup>2,5,8,9</sup> should leave no doubt that: (1) initially the number of DNA breaks are identical in oxic and anoxic irradiation and (2) rapid repair, involving DNA polymerase I and probably ligase, does exist in cells and preferentially rejoins anoxic-type breaks. This could explain the differ-

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<sup>3</sup> Raff, R. A., and Mahler, H. R., *Science*, **177**, 575-582 (1972).

ences in the number of breaks in cellular DNA.

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JOHANSEN AND BOYE REPLY—We believe Srivastava's conclusions<sup>1</sup> are based on misinterpretations of circumstantial evidence. First, it has long been known that when bacteriophages are irradiated extracellularly the survival of their plaque-forming ability is identical in oxygen and in nitrogen. When irradiated in the presence of sulphhydryls, however, the survival in nitrogen is increased, and becomes higher than in the presence of oxygen<sup>2</sup>. This suggests the importance of sulphhydryl compounds in anoxic protection. The finding that the yield of DNA strand breaks is similar in oxic and anoxic conditions when phage  $\lambda$  is irradiated extracellularly in the absence of sulphhydryls<sup>3</sup> is as expected, and within the framework of classical radiation biology.

Second, when irradiated intracellularly, in normal physiological condi-

bacteria heat shocked before irradiation<sup>4</sup>; (3) bacteria kept at pH 8.6 (ref. 8). During these treatments, the cells become leaky and considerable amounts of sulphhydryls are lost into the suspending medium<sup>5</sup>; (4) Srivastava's own experiment with intracellular phage  $\lambda$  (ref. 3). We noticed that in this experiment the bacteria were suspended in 0.01 M  $\text{MgSO}_4$  before irradiation, and wondered whether this hypotonic treatment had any effect on the level of endogenous sulphhydryls in the bacteria. As can be seen from Table 1, suspending bacteria in 0.01 M  $\text{MgSO}_4$  leads to a loss of a considerable amount of sulphhydryls from the cells. Small contaminating amounts of oxygen—if present—will increase the 'anoxic' yield of radiation-induced DNA strand breaks in these cells.

Third, experiments in which phage  $\lambda$  is irradiated extracellularly in oxygen or in nitrogen in the absence of sulphhydryls, and the DNA analysed 10 min after infection of a repair-proficient strain are very complex, and interpretation is not unambiguous. In conclusion, recent experiments<sup>4,5</sup> tend to support the view that the oxygen effect in radiation-induced DNA strand breakage is due to radiochemical reactions, rather than to preferential enzymic repair.

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Table 1 Effect of hypotonic treatment on cellular sulphhydryls

Treatment*	Sulphhydryls in cell-free supernatant (mM)	Sulphhydryls in TCA extract (mM)
Phosphate buffer, pH 6.8	<0.05	2.04
0.01 M $\text{MgSO}_4$	1.15	1.12

\*Cells of *E. coli* K12, strain AB 1157, from a 100-ml log-phase suspension were collected by centrifugation and washed in phosphate buffer<sup>1</sup> at pH 6.8. The cells were resuspended in 0.3 ml of the same buffer or in 0.3 ml 0.01 M  $\text{MgSO}_4$ , kept for 10 min at room temperature and the concentration of sulphhydryls measured in the cell-free supernatant<sup>5</sup>. The bacteria were suspended in 0.3 ml trichloroacetic acid (TCA) at 0.3 M, kept at room temperature for 10 min and the concentration of acid soluble sulphhydryls measured in the cell-free extract.

tions, a higher yield of strand breaks is generally found in oxic than in anoxic conditions, both for chromosomal and phage DNA. This is true even when the experiments are performed so fast that the ligase could rejoin, at the most, one of a hundred DNA strand breaks formed<sup>4,5</sup>. We are aware of four types of experiment where the anoxic yield of DNA strand breaks is increased to—or nearly to—the oxic yield: (1) bacteria treated with the sulphhydryl-binding agent *N*-ethyl-maleimide (NEM) before irradiation<sup>6</sup>. This sensitisation is reversed by strict anoxia<sup>7</sup> and is believed to be caused by increased sensitivity to small contaminating amounts of oxygen in cells with low levels of endogenous sulphhydryls<sup>8</sup>; (2)

size or development...". Chang *et al.*<sup>2</sup> investigated the well-known asymmetry of the scrotum in man and showed that in right-handed subjects the right testis tended to be higher, whereas the converse applied in left-handed subjects. To investigate whether this was simply due to the greater weight of the left testis in right-handed subjects they measured the weight and volume of the testes in (presumably mainly right-handed) cadavers and found, paradoxically, that the right (that is, the higher) testicle was also the heavier and of greater volume, a result in accord with Mittwoch and Kirk's foetal data<sup>1</sup>.

Interest in testicular asymmetry may however be traced back much further. Winckelmann<sup>3</sup> in 1764 commented that: "Even the private parts have their appropriate beauty. The left testicle is always the larger, as it is in nature;". He went on, however, "so likewise it has been observed that the sight of the left eye is keener than the right", an observation which, to my knowledge, has not been confirmed.

To test Winckelmann's claim, I observed the scrotal asymmetry of 107 sculptures, either of antique origin or Renaissance copies, in a number of Italian museums and galleries. Table 1 shows that although the ancient artists were correct in tending to place the right testicle higher, they were wrong in so far as they also tended to make the lower testicle the larger: we may postulate that they were also using the common-sense view that the heavier ought to be lower. Although Winckelmann's observations of antique sculpture were correct, his observations of nature are clearly in error.

The reason for the artists placing the right testicle higher than the left is not clear. It may reflect the true observed state of things, but it may also be a function of Greek left/right symbolism, in which right and male, and left and female were regarded as equivalent, and thus for instance, the male child was presumed to come from the right (and thus higher?) testis, and vice versa for the female child<sup>4</sup>.

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## Scrotal asymmetry in man and in ancient sculpture

MITTWOCH and Kirk<sup>1</sup> have claimed that "Right and left mammalian gonads do not usually differ noticeably either in

Table 1 Analysis of the scrotal asymmetry of 107 ancient sculptures

Side of larger testicle	Side of higher testicle			Total
	Left	Equal	Right	
Left	2	7	32	41
Equal	8	19	17	44
Right	17	1	4	22
Total	27	27	53	107

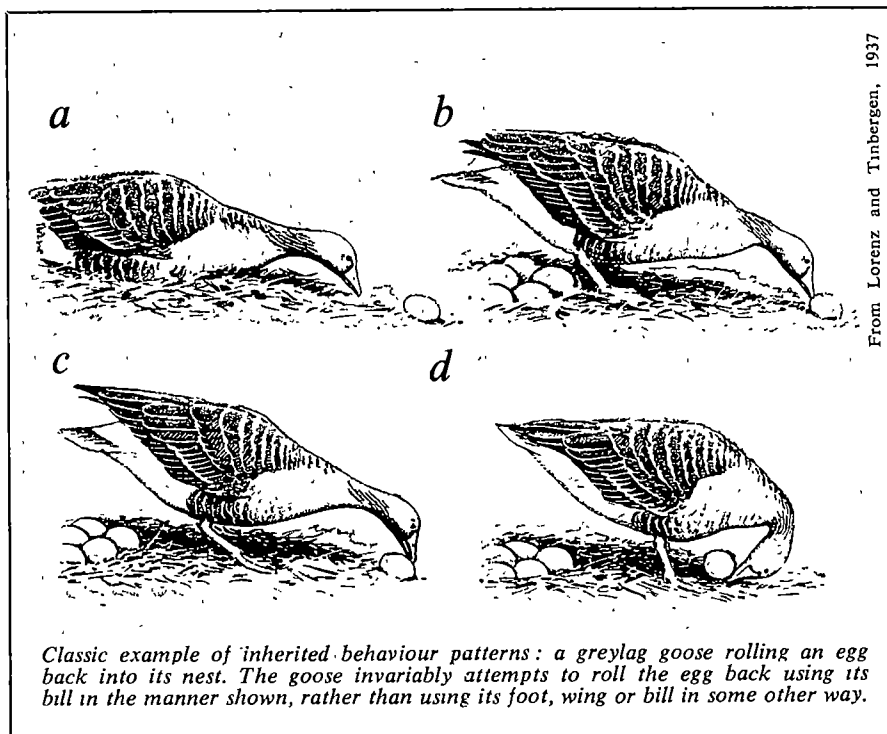
# reviews

## Optimum strategy for perpetuating genes?

ANY textbook on behaviour which attempts to go beyond the single topic monograph, presents its author with formidable problems concerning the selection and organisation of material. Behavioural studies may have their origins in fields as far separated as neurophysiology and ecology. Brown's book\* has evolution as a unifying theme, and indeed behaviour itself is not always its main focus, for it contains a great deal of material pertinent to the mechanisms of evolution which would not normally be found in a behavioural text. It is very much a product of the new sociobiology school, and none the worse for that. We have certainly gained many fresh insights into animal behaviour from a consideration of the selective forces which operate on individuals with respect to their interactions with others of their species.

Brown operates very skilfully on the borderlines between ethology, population biology and genetics, and he is highly competent in all of them. His selection of literature is good and experimental results are described, and often illustrated, in sufficient detail for the reader to understand their significance fully. On those topics where I am competent to judge the adequacy with which a controversy is being discussed, Brown misses very few of the points and this is a rare quality in textbooks.

There are some penalties to be paid for being so comprehensive. The book is very long and the choice of more purely behavioural topics for inclusion seems rather arbitrary. Brown begins with a brief prologue which I found rather odd. He lays stress on diversity between species and constancy within species without once mentioning change. Indeed almost at the outset he states that species "... tend to persist



From Lorenz and Tinbergen, 1937

*Classic example of inherited behaviour patterns: a greylag goose rolling an egg back into its nest. The goose invariably attempts to roll the egg back using its bill in the manner shown, rather than using its foot, wing or bill in some other way.*

virtually the same for generation after generation." True, but not an idea I would choose to emphasise at the start of a book on evolution. The changes which natural selection can elicit from populations form the bulk of the book which follows.

The book is organised into six sections of very diverse proportions. Thus the first two on phylogeny and genetic basis of behaviour have but single chapters. The former is a brief but fresh survey of the possibilities for analyses of the phyletic descent of behaviour within and between groups. The genetics chapter is less satisfactory, although it provides a reasonable review of the different approaches in behaviour genetics (with however, no reference to the quantitative analysis of the genetic architecture of behavioural traits). Nevertheless this chapter seems very isolated from the rest of the book because our knowledge of the inheritance of behaviour does not yet match up to some of the questions which evolutionary studies force us to ask.

The book's real qualities emerge in sections 3 and 4 on social organisation and communication. Brown gives an excellent account of the modern sociobiological approach to animals in groups. He deals with the factors which determine the nature and size of groups and goes on to discuss mating systems and aid-giving behaviour of all

types. He presents most of the arguments very clearly in depth and I find it the more strange that Wynne-Edwards' ideas on group selection and the origins of social behaviour are given such brief treatment. They seem to me of great importance not because they are right (I think they clearly are not) but because of the effect they had on the course of sociobiology. The controversy which surrounded Wynne-Edwards' theories led to a great deal more attention being focused on the individuals within the group. This was vital in the development of the modern synthesis of behavioural and evolutionary concepts.

Will all social behaviour, including that which gives aid to others, turn out to be an optimum strategy for perpetuating one's genes? Certainly we need to know as much as possible about the genetic relationships within groups. Brown provides a very good review of the evolution of behaviour in relation to kin selection. He manages to retain clarity on some complex issues without oversimplifying.

The section on communication is more purely behavioural but the material provides some of the classic stories in the evolution of behaviour. Just as did Marler and Hamilton in their 1967 textbook, so Brown by skilful choice of examples manages to make the whole subject fresh. There is also a

\**The Evolution of Behaviour*. By Jeram L. Brown. Pp. xix+761. (W. W. Norton: New York, 1975.) \$15.95.



## Good but expensive fare

*Introduction to Physiology.* By Hugh Davson and M. B. Segal. Volume 1: Basic Mechanisms, Part 1. Pp. xii+561. Volume 2: Basic Mechanisms, Part 2. Pp. xi+481. (Academic: London and New York, June 1975.) £6.80 each part.

THESE two volumes are the first to appear in a series of five. A reviewer thus faces a similar dilemma to a diner passing judgement on a complete meal after sampling only the soup and fish. In this instance, however, the renown of the chef de cuisine, Dr Hugh Davson, is such that it is only necessary to decide for what type of clientele he is catering to predict what is in store. It turns out that, along with his co-author, Dr Segal, he is providing good, solid and easily digestible fare without too many sophisticated trimmings but also without succumbing to the hazards of oversimplification.

Volume 1 deals, in the opening chapters, with cellular physiology and includes a good deal of material usually classed as biochemistry—for example, the tricarboxylic acid cycle and protein synthesis. Such fundamental topics in general physiology as osmosis and the genesis of bioelectric potentials are also covered and up-to-date descriptions of ultrastructural findings related to cellular function and interactions are provided. Some elementary thermodynamics is also included but, although simple equations are used, the approach is essentially descriptive. The concept of chemical potential, although it receives a passing mention, is not seriously developed and the section on

entropy is one of the few instances in which the exposition is a trifle confusing, if not actually misleading. The remaining chapters are devoted to haemodynamics, respiratory exchange, in testinal absorption and renal function, all of which are treated in considerable detail. The second volume is concerned with the nervous system (chiefly the peripheral and spinal divisions), hormonal control (discussed in rather general terms), the effector systems (muscles and glands), defence mechanisms (mainly haematology and immunology) and reproduction (including some 20 pages on lactation).

The overall presentation is clear and commendably free from errors, although an occasional slip such as the heading "carbonate" over a section evidently devoted to carbamate could be tiresome to a beginner. The illustrations are of high quality and generally well chosen, but it seems odd to reproduce a typical Gamble diagram and attribute it to a recent review, and to opt for an oxygen dissociation curve published in 1958 which adds little to the one published by Bohr and Krogh in 1904. Students with hardly more scientific preparation than a nodding acquaintance with chemical formulae and graphs, should find these books comprehensible. After mastering the five volumes (if the succeeding ones resemble the first two) such students will be not only introduced to but thoroughly at home in physiology. Some, however, may find this protracted initiation and the entrance fee (presumably in five instalments of £6.80 each), which goes with it, too disheartening and will feel tempted to seek another portal of entry to the subject.

R. V. Coxon

very good account of the evolution of display movements although the motivational analysis of displays which has played such a large part in the development of this area of ethology, and which has strong evolutionary implications, is mentioned only in brief.

For the final sections of his book Brown chooses some topics on what he calls "the physiological basis of species constancy and species diversity in behaviour." The chapters on reflexes and fixed action patterns do fit the plan, but he goes on to deal with sensory systems, migration and circadian rhythms. They are all good, modern accounts, but I fail to see where they fit into Brown's evolutionary scheme. I have similar problems with the contents of the final section on development, in which the behaviour of embryos is covered in detail, but there is no mention of learning. The concluding chapter on bird song, however, does provide an opportunity to discuss the evolution of

development itself. The birds provide such a good range of ecological and social variants which we can now begin to correlate with the manner in which their song develops.

So this review ends as it began, quibbling about the selection of material. Brown's evolutionary theme has sufficient strength to give a solid backbone to his chapters even though all of us would leave some things out and include others. I think this is a distinguished textbook. In Britain it is probably too long for most undergraduate courses, but it can nevertheless unhesitatingly be commended as a book for students to consult. I defy anyone to browse in the central chapters without having their attention riveted by some elegant and fascinating example of the manner in which their behaviour has come to equip animals for survival and I can pay Brown no higher compliment.

Aubrey Manning

## Cryogenics

*Heat Transfer at Low Temperatures.* (The International Cryogenics Monograph Series.) Edited by Walter Frost. Pp. xiv+382. (Plenum: New York and London, 1975.) \$42.00.

THIS book "is intended to enhance the knowledge of the thermal design engineer faced with solving heat transfer and fluid flow problems at low temperatures." The temperature range which is covered lies, in effect, between room temperature and 1K: thermal transfer problems peculiar to lower temperatures than this are not described at all. The book consists of 15 chapters, in reality separate articles prepared by 20 different contributors.

The first two chapters deal effectively with 'classical' thermal transfer through conduction and convection. The second, and main, part of the book consists of ten chapters devoted entirely to two-phase phenomena of various sorts. Topics such as nucleate pool boiling, film boiling, two-phase flow, and the condensation of gases on cryogenic surfaces are treated in considerable detail. The third part consists of two chapters on topics which, presumably, did not seem to the editor to fit in conveniently anywhere else: one on radiative heating, with particular reference to the influence of cryodeposits on the cold surface; and a final chapter on thermal transfer to HeII.

No attempt has been made to standardise nomenclature or units between the different chapters: pounds and feet coexist uneasily with kilograms and centimeters; BTU with ergs and joules; and Kelvins with degrees Centigrade and even Fahrenheit. The remark on the dustcover about "surveying the literature to date . . ." was no doubt accurate at the time of writing. While three or more years may perhaps be a fairly normal publishers' gestation period these days, it seems rather a pity that more could not have been done to update the book at the proof stage: this would, at least, have enabled some modification of the amusingly anachronistic remark in the final chapter to the effect that liquid 'He and the superconducting electron gas are the only known superfluids.

The book does contain a great deal of information, however, so that, in spite of these minor shortcomings, it should certainly be useful to aspiring designers of rockets, space simulators, superconducting generators and other large scale cryogenic machines involving the flow or storage of liquified gases.

P. V. E. McClintock



## Molecular rearrangements

*Isotopes in Organic Chemistry*. Volume 1: Isotopes in Molecular Rearrangements. Edited by E. Buncl and C. C. Lee. Pp. xvi+301. (Elsevier Scientific: Amsterdam, Oxford and New York, 1975.) Dfl.100; \$41.75.

LABELLING experiments have often been crucial to the development and understanding of modern organic chemistry and few will question after reading this book that many mechanistic studies would be considerably harder, if not impossible, without the availability of isotopic techniques. Nonetheless, good texts and reviews of this field have been thin on the ground; this series, of which the pre-

sent volume is the first, should fill an important gap in the chemical literature.

Both carbonium ion (26 pages) and carbanion (105 pages) rearrangements are given another airing by N. C. Deno and D. H. Hunter, respectively, and pericyclic reactions (33 pages) are reviewed by W. R. Dolbier. J. S. Swenton's contribution concerns photochemically initiated rearrangements (51 pages) and J. L. Holmes deals with rearrangements occurring alongside mass spectral fragmentations (73 pages). As one might expect from these particular authors, of whom all have made significant contributions to their specialised topics, their articles are well-written from an authoritative standpoint, and all seem to cover the literature up to 1973. Generally, the

authors have written a fairly descriptive text of the kind usually appreciated by organic chemists and have attempted (with varying success) to relate the isotopic results to other mechanistic criteria. The editors have avoided the obvious pitfall of allowing repetitious theoretical introductions to each chapter, so the amount of worthwhile information is high.

This book (and probably the series, too) is written for the specialist with a deep interest in organic mechanistic studies. Elsevier might seriously have considered making each chapter available separately, particularly as the contents of the present volume span such a wide range of interests. Nonetheless, I strongly recommend the text as a valuable addition to any chemical library.

B. C. Challis



*Illustrations taken from British Botanical and Horticultural Literature before 1800. By Blanche Henrey. Three Volumes. Pp. 1,128. 32 colour plates, 162 pp. black and white illustrations. (Oxford University: London, 1975.) £70. A comprehensive account of books and pamphlets on botany, gardening, horticulture, arboriculture and silviculture from the sixteenth to eighteenth centuries. Left, Helleborus niger L., Christmas Rose. Drawn and engraved by James Sowerby. From Medical Botany (1790), Vol. 1 by Woodville. Right, Lillium martagon L., Martagon or Mountain Lilly. Engraving from The Compleat Florist (1740).*



## Neoplastic development

*Neoplastic Development*. Volume 2. By Leslie Foulds. Pp. xiv+729. (Academic London and New York, August 1975). £16.80; \$43.50.

LESLIE FOULDS spent most of his working life in experimental cancer research, but his interest in cancer extended beyond the laboratory. His object in writing this book is perhaps best given in his own words:

In any branch of science it is important to confront inferences from laboratory experiments with the stark realities of natural phenomena... It is especially important... when the growing estrangement between clinical practice and laboratory research is leading to grave doubts about the ability of laboratory research, as now conducted, to make any substantial contribution to the alleviation of human suffering attributable to neoplastic disease. Writing from the standpoint of a medically qualified experimental pathologist with a long-standing interest

in both developmental biology and medical practice but with no direct participation in either, I hope to illustrate in this volume (Volume 2) the complimentary and essential, but diverse contributions, of biological theory, laboratory analysis and clinical experience of human disease...

Volume 1 provided a general theory, basically that neoplasia is a developmental process akin to normal development but differing from it in some important respects, still to be defined. In Volume 2, he considers special cases of neoplastic development in man, and animals, and discusses the biological and medical implications. After a brief general introduction, there are large sections on neoplasia of the skin, mammary neoplasia in laboratory animals and human breast cancer. Shorter sections deal with cancer of uterine cervix, urinary tract, lungs, liver and some endocrine glands, and there are two brief sections on neoplasia of unknown pathogenesis and virus tumours. The section on skin is the most extensive and is used to establish general

principle concerned with the initiation-promotion theory and with tumour progression. The latter is illustrated by descriptions of the disease process in man and in the experimental animal, and a discussion on aetiology. A similar approach is used in the other sections.

Like its predecessor it is a valuable work and few of those interested in cancer in general or in one of the special fields discussed will fail to learn something of value. It will teach the clinician something of experimental cancer research and the research worker a little about human cancer, but in a major objective—a more rational approach to the clinical management of the patient—it must be regarded as a gallant failure, not through a fault of the author but through our ignorance. It is sad that the author should have died from cancer shortly before the book was published, and before he completed the final chapter summarising his views and general conclusions.

L. M. Franks



## Bold perspective

*Ion-selective Electrodes.* (Cambridge Monographs in Physical Chemistry Volume 2.) By Jiri Koryta. Pp. 207. (Cambridge University: Cambridge and London, August 1975.) £10.50.

THE development and application of ion-selective electrodes (ISEs) based on membrane electrochemical principles have been, with comparable activity in liquid chromatography, the two most rapidly advancing areas in chemical analysis and continuous monitoring. Since 1965 improved or new measurements have become possible which have not only given impetus to analytical potentiometry, but have a broad impact in diverse areas as environmental science, physiology, clinical chemistry and biophysics. In fact one difficulty in following progress in the field is the widespread use of ISEs

and the large number of specialist journals in which results appear. ISEs are serving the fundamental purposes of making analytical chemistry visible in other branches of chemistry, physics, medical and health sciences and the converse effect of broadening the interests of analytical chemists in interdisciplinary problems and projects.

To write a book at midstream in a rapidly moving field requires courage, and to achieve a sense of perspective on the past and possible future developments is most unusual. Koryta's new volume is well organised and executed, and contains clearly presented fundamentals and applications drawn from all fields touched by ISEs. Jiri Koryta is a well known electrochemist, a major disciple of Nobel Laureate J. Heyrovsky and an exponent of the polarographic method. His outstanding contributions to theory and practice of coupled, homogeneous-heterogeneous electrode processes, complex ion equilibria and adsorption effects make him an ideal commentator on the field of ISEs and membrane processes. The primary strength of the book is the mature overview given on the topics of diffusion-migration and the interfacial processes which underlie the potential sources for fixed site, mobile site and neutral carrier classes of membrane electrodes. At the same time practical, analytical methods are tabulated and current topics such as continuous monitoring and use of computer-controlled titrations are described.

Only on hindsight can one determine key, definitive volumes on a given speciality area. Because of the continuing growth in this field, all previous books, although useful for reference, are clearly premature. This new book unfortunately must suffer from the same limitations: new fundamental studies on response mechanisms, time-dependence of responses and applications are still appearing in current literature at a rapid rate. This volume contains literature citations only as recent as 1973 and is an expansion of Koryta's excellent 1972 review article. Nevertheless, this book is the finest, comprehensive work published to date.

**Richard P. Buck**

## Climatic change

*Ice Ages: Ancient and Modern.* (Proceedings of the 21st Inter-University Congress, January 1974.) Edited by A. E. Wright and F. Mosley. Pp. 320. (Seel House: Liverpool, 1975.) £12.00; \$33.00.

THIS book is advertised as suitable for geology and geography students "from sixth form to final year undergraduate level"; but unless the sixth formers of

today are markedly more able than those of my generation it would seem better suited to students of Earth sciences at a rather later stage of their academic careers, including those active in research. For such people, the book will be of most value to those who have had a less formal education in geography and geology at an earlier stage. It provides a good quick guide to geology and timescales, to processes of sedimentation and to glacial tectonic structures, before moving on to discuss climate and climatic change in more detail.

There is one exceptionable statement early in the first section ("Introduction to the Quaternary") in which F. W. Shotton refers to ice ages occurring when "... snowlines extend outwards from the poles, ice sheets expand, mountain glaciers push forward ..." and makes no reference to the recent work which suggests strongly that ice ages can occur much more quickly than this older picture suggests, with snow and ice cover building up over a wide area of the northern hemisphere in a few years or decades. Otherwise, the various contributions (which are based on lectures given at Birmingham University in January 1974) provide a good overview of how geological and other evidence (pollen analysis, studies of fossil beetles and so on) provides an indication of how climate has fluctuated.

But the discussion of the causes of such changes is less satisfactory, and the volume also suffers from being almost entirely concerned with events in north-western Europe, particularly Britain. It is inexcusable, in a book of this length, to dismiss variations caused by changes in the Earth's orbit and inclination in less than a page and by volcanic dust in three lines. There is a preponderance of geological jargon in some of the contributions, and most of the contributors share the geologists' preference for talking in terms of the named geological periods of time, rather than in years b.p. These deficiencies are balanced to some extent by the copious references and by a summary from the editors putting the various contributions into perspective. In this case, the summary is particularly valuable and does not become bogged down by attempts at verbatim reporting of question and answer sessions. The importance of this kind of research, and the slightly parochial inclination of this book, are highlighted by one titbit from the summary: "even today it would only require an average temperature of 2 °C to [produce] glaciers on Ben Nevis".

Those searching for more answers in the study of climatic changes will be stimulated by this volume.

**John Gribbin**

## Nuclear structure

*Structure of the Nucleus.* By M. A. Preston and R. K. Bhaduri. Pp. xiv+693. (Addison-Wesley: Reading, Massachusetts and London, August 1975.) Cloth \$29.50; Paper \$19.50.

FOR more than a decade Preston's *Physics of the Nucleus* has been used by nuclear physicists as a comprehensive and reliable reference book. Its essential theme is the attempt to derive the properties of the nucleus from our knowledge of the nucleon-nucleon forces. This can be done to some extent, but in many respects we still have to rely on phenomenological models. The years since 1962 have seen many important advances, and while progress has been made in some areas the problem is now seen to be more complicated than was previously thought, particularly because of the importance of many-body forces. To deal with this situation Professor Preston, now joined by Dr. Bhaduri, has thoroughly revised and re-written the chapters dealing with nuclear structure, and since this is already as long as the whole book before revision the chapters on nuclear reactions have been omitted. The plan is very similar and successive chapters deal with the constituents of nuclei, internucleon forces, nuclear moments, nuclear shapes and sizes, and nuclear binding energies. The second part is devoted to nuclear models, correlation in nuclear matter, collective nuclear motion, and Hartree-Fock and particle-hole calculations. The final part covers  $\alpha$  radioactivity and fission. The standard of the original volume is amply maintained, and the new version will be a valuable and much-used text for years to come. **P. E. Hodgson**

## Human tumour lines

*Human Tumour Cells in Vitro*. Edited by Jørgen Fogh. Pp. xix+557. (Plenum: New York and London, 1975.) \$44.50.

THIS book, a collection of 16 papers on human tumour cell culture, has appeared at an appropriate time. Belatedly research laboratories are beginning to show an increasing interest in cancer in man and at the same time there is an increasing, if somewhat misguided, pressure from society to limit suffering in laboratory animals, particularly since it cannot or will not limit suffering among men. Consequently *in vitro* systems, especially those using human tissue, are becoming increasingly popular. As in any branch of science the value of a particular technique depends on the questions it is being used to answer. If cell cultures are being used for virus production or cytotoxicity testing (for example), the nature of the cells is unimportant provided they serve the purpose of the experiment. If the experiments are concerned with the nature and behaviour of the cells themselves a more critical approach is required.

It is important to know the precise origin of the cells and the variations in structure and function which they may have developed as a consequence of establishment *in vitro*. This was well known to the early practitioners of the art of cell culture—all of whom were biologists or physicians accustomed to studying the whole individual—but with the expansion of cell and molecular biology, concerned principally with isolated cells, and an era when living cells could be ordered and supplied 'off the shelf' by laboratory supply houses, these basic facts were often forgotten. Although the whole universe may be contained in a grain of sand the whole of biology is not contained in a HeLa or BHK cell. Why for example is it possible to maintain such a small proportion of tumour cells in culture as permanently established cell lines? How can tumour cells *in vitro* be identified with certainty and distinguished from mesenchymal cells arising from the non-malignant stroma of the tumour? *Human Tumour Cells in Vitro* considers some of these problems.

Apart from a valuable description of 31 new human tumour lines by the editor and Dr Trempe, most of the information is available elsewhere to those with access to a good library but for the others it provides a useful survey of methods and media which have been used for the establishment of human tumour cell lines *in vitro*. There are also sections on cytological characterisation, chromosome abnormalities and

ultrastructure and on the search for viruses in human cancer cells.

L. M. Franks

## Pineal physiology

*Frontiers of Pineal Physiology*. Edited by Mark D. Altschule. Pp. xii+269. (MIT: Cambridge, Massachusetts and London, 1975.) £8.75.

It would be a pity if this book were read as an entrée to the new and exciting realm of pineal physiology; its title stakes a claim which is wholly unjustified by its contents, a collection of papers (some reviews, others research reports, yet others an uneasy mixture of the two) uneven both in length and quality. Five years have passed since the conference on which it is based was held, and this latent period is all too obvious in places.

The intense enzymatic activity of the pineal, and the way this is changed by either natural or experimental lighting are characteristic features. Three chapters survey this area, with varying degrees of thoroughness. A firmer editorial hand might have improved the organisation of this part of the book, and reduced the considerable overlap between individual chapters; much of these have also appeared in other reviews by the same authors.

Although studies on pineal function are still dominated by the discovery of melatonin, increasing attention is now being given to pineal polypeptides, some of which seem to resemble those found in the hypothalamus and pituitary; two research reports deal with these substances. P. H. Sampson contributes a brief survey on the way the pineal may be involved in various categories of behaviour, most of which is a lengthy report of an experiment showing that pinealectomy somewhat reduced maternal behaviour. We are left wondering what this might mean in terms of pineal function, for no interpretation is offered.

The most intriguing, and inevitably the most controversial, part of the book explores the possible association between malfunction of the pineal and schizophrenia. The large amount of 5-hydroxytryptamine in the pineal and the suspicion that this monoamine is involved in schizophrenia make the link tempting (although drugs ameliorating this condition are generally dopamine blockers) M. D. Altschule contributes a chapter which is too brief either to inform adequately or to convince in which it is said that administering pineal extracts improves abnormalities in carbohydrate metabolism in schizophrenics, although how these are related to the disease is unclear. L. B. Bigelow describes the results of a trial in which schizo-

phrenics were given daily injections of melatonin-free pineal extract. In spite of a curious method of measuring the patient's mental state, an uneven baseline and coincident changes in phenothiazines, the results of at least one case suggest the need for a more comprehensive study. Dr Bigelow quotes the isolation from the pineal of a substance inhibiting the formation of dimethyltryptamines, long known to be hallucinogenic. What a pity his study didn't concentrate specifically on these symptoms.

This book represents the frontiers of our knowledge indistinctly, and the signposts to the future are hard to discern from reading it. J. Herbert

## Xylem strategy

*Ecological Strategies of Xylem Evolution*. By Sherwin Carlquist. Pp. xi+259. (University of California: Berkeley, Los Angeles and London, August 1975.) £6.85.

THIS book is profoundly disappointing. The subject matter does have general interest. Do such features as the lengths of tracheids and vessel elements, the shapes and sizes of these cells in transverse section and the shapes and sizes of the pits between these cells have any significance in the adaptation of plants to their habitats? Is the disposition of parenchyma and fibres within and around the xylem of adaptive significance? Any satisfying book would have to start from a statement of theory regarding the functional effects of these features, together with such experimental evidence as is available. The author offers no such opening statement, although relevant comments are scattered through the book. He considers each major vascular group in turn, seeking an exact harmony between certain arbitrarily chosen xylem features and the habitat of the plant. The whole account is riddled with unsupported statements, special pleading and illogical handling of facts and ideas. The author never deals adequately with the phenotypic as opposed to genotypic differences between plants; slower-growing individuals generally have shorter, narrower xylem elements, but is this feature necessarily adaptive? There are some suggestive correlations of habitat with xylem cell form, but in respect of most of the detailed differences in xylem type one is bound to agree that "the logical conclusion to be drawn is that a variety of peculiar types are functionally quite effective" (p23). This book should have been condensed into a review article.

P. J. Grubb



*Nuclear Quadrupole Resonance in Chemistry* By G. K. Semin, T. A. Babushkima and G. C. Yakobson. Pp. X+517. (Halsted, Wiley: New York and London; Israel Program for Scientific Translations: Jerusalem, October 1975.) £24.75.

THIS is a reasonably quick translation of an original published in the Russian in 1972. It does, however, have an inordinate price, considering that it has not even been set in normal print, but rather is the product of an electric typewriter. It is greatly to the credit of the authors that notwithstanding these shortcomings one is able to recommend it unreservedly to researchers in nuclear quadrupole resonance (NQR) spectroscopy, who will find 300 pages of data compilation invaluable (particularly because they contain so much from the Russian literature). The greatest emphasis is on the application of the technique to chemical problems and although the expert is so well catered for the authors have provided an adequate introduction to the technique and to the basic features of its theory. Any chemist reading the chapters which precede the data tables would acquire a sound feeling for the possible applications of NQR in his own field of research. What is not dealt with in detail, deliberately, is the quantum-mechanical calculation of coupling constants but this is no real shortcoming in a book which describes the chemical applications uniquely well.

The outstanding Russian contribution to this field of research is presented with modesty in a fascinating book.

**K. A. McLaughlan**

*The Living Ocean: Marine Microbiology.* (Biology and Environment) By E. J. Ferguson-Wood. Pp. 146. (Croom Helm: London, September 1975.) £5.50.

I ENJOYED reading this book which is about the structure, ecology and economics of marine prokaryotes and protists. It was intended as a personal and selective account and has been rescued from an early draft prepared shortly before the author's death. It is therefore obviously open to some criticisms of approach and incompleteness. Ferguson-Wood's enthusiasm and involvement, however, are evident from the text which may be of most use as an infectious introduction to the subject. The book can be recommended to the beginner or layman, but cannot be regarded as an alternative to other accounts of microbiology, including the author's previous books, for more serious

students. Rather few references are given (in the guise of notes at the end of each chapter), some of these are rather trivial and more obvious important ones are omitted. The short title given on the cover and binding does not fairly indicate the book's content otherwise it is quite well produced with few minor errors —although some of the diagrams are poorly prepared. Professor Ferguson-Wood certainly contributed to the main course of marine microbiology; this book is a rather expensive aperitif.

**P. J. S. Boaden**

## Books brief

*Physiology of Physical Stress: A Selective Bibliography, 1500–1964.* By Carleton B. Chapman and Elinor C. Reinmiller. Pp. vii+369. (Harvard University: Cambridge, Massachusetts and London, 1975.) £8.25.

THE compilers of this bibliography have selected 2,800 references concerned with some aspect of the physiology of physical stress. Physical stress seems to mean physical exercise or physical activity, to judge from the preface. References dealing with 'basic physiological work on physical stress' are included but 'extensions were made into clinical areas that had basic implications'.

The word 'stress' is notoriously difficult to define, and for that reason is best avoided whenever possible. The title might well have been 'The physiology of physical exercise'. It is difficult to determine why the authors include particular papers but not others. The word 'basic' is used five times in the third paragraph of the preface where an account is given of the identification of 'basic' articles. No doubt there would be considerable individual differences in the interpretation of 'basic' and thus decisions to include or reject must be to some extent arbitrary. All the same, I was surprised at the omission of Marius Nielsen's famous paper on the regulation of body temperature during physical exercise, and no reference is made to Adolph and *Physiology of Man in the Desert*. There are some other equally surprising gaps and some curious inclusions.

Nevertheless, one must applaud the determination required to prepare a bibliography which, in spite of some reservations, I have found useful.

**O. G. Edholm**

*Electrode Kinetics* (Oxford Chemistry Series.) By John Albery. Pp. xii+184. (Clarendon: Oxford; Oxford University: London, June 1975.) £5 00.

ELECTRODE kinetics is a difficult subject, especially for the beginner, and so a new book setting down the fundamental ideas in a clear, lively and occasionally humorous manner is welcome. Many will also see this particular book as an original contribution to the subject. It is suitable for specialist first degree courses in electrode kinetics but especially for new research workers in the field. There is also much to interest the expert, with many new angles on old problems. The subject is presented in a way which should be intelligible to the beginner. Even so, the amount of detail is considerable and many of the finer points are discussed. Inevitably there is much mathematics, but the reader is helped through this by numerous sketches of the important functions. The choice of much of the material reflects the author's own interests. There is an excellent chapter on the double layer and an illuminating one on the theory of electron transfer which should interest a wider audience.

**D. R. Whitehouse**

*Enzyme Induction.* (Basic Life Sciences, Vol. 6.) Edited by D. V. Parke. Pp. xii+328. (Plenum: London and New York, 1975.) \$32.50. THE title of this interesting book, which covers a Colloquium held at the University of Surrey in June 1972, is rather deceiving. "Enzyme induction" has been coined for a phenomenon involving *de novo* synthesis of a specific protein in response to a specific signal in the environment, such as the synthesis of  $\beta$ -galactosidase or tryptophanase by *Escherichia coli*, in response to the presence of a  $\beta$ -galactoside or tryptophan in the growth medium.

Except the first chapter dealing exactly with phenomena of this type, the rest of the book deals mainly with the effects of drugs and steroids on the appearance of several enzymes at the cellular or developmental level. This appearance is not necessarily a reflection of a *de novo* synthesis, but may be the result of a decreased degradation attributable or not to a stabilisation of the enzyme studied.

The various contributions of the book will prove very useful to those interested in the mechanisms of biological regulation and their possible roles in health, ageing and disease. The chapter on induction of drug-metabolising enzymes present in liver endoplasmic reticulum is of special interest.

**Georges N. Cohen**

# obituary

**James Gray** was born in London on October 14, 1891. His parents were both Scottish and he always felt himself to be an expatriate Scot. After early education at the Merchant Taylor's School he became first a Scholar and later a Fellow of King's College, Cambridge. Shortly after his election, his academic career was interrupted by the First World War, in which he served, with great distinction, as a Captain in the Queen's Royal West Surrey Regiment. He was awarded the Military Cross and had conferred upon him, by Marshall Foch in person on the field of battle, the Croix de Guerre with palms. After the war he returned to Cambridge to resume his Fellowship and soon to be married, in 1920, to Norah Christine King, who survives him. Not long after this he was appointed Lecturer, later Reader, and in 1937 he was elected to the Chair of Zoology which he occupied until he reached the retiring age, in 1959. During retirement he continued to reside in Cambridge, where he died peacefully in his own home on December 14, 1975.

Gray's first research was concerned with the fertilisation and early development of echinoderm eggs, but already he was a cell biologist rather than an embryologist. It was primarily his interest in cells that led him on to the study of ciliary movement on which he quickly became the world authority, as was recognised by his election to Fellowship of the Royal Society in 1929. After writing a notable book, *Experimental Cytology*, he made up his mind to leave this field; he felt that, using living cells, means were not then available to formulate meaningful questions and to obtain clear-cut answers—and he was not interested in dead cells. In deciding to move into the field of locomotion he was no doubt influenced by the circumstance that he had developed the techniques of cinematography for use on cilia, and these techniques could be applied to other types of movement. Animal locomotion occupied him over the next 25 years, bringing him the award of the Royal Medal of the Royal Society in 1948, and it is for his work in this field that he will be long remembered. His calculation that the power required to drag a dolphin's dead body through water is greater than the power available from its muscles has become

widely known as Gray's Paradox. From the swimming of dolphins to the swimming of spermatozoa, from the movement of snakes to the movement of minute nematode worms he explored the undulatory mode of propulsion, and also investigated, in collaboration with H. W. Lissmann, the modes of terrestrial locomotion seen in amphibia. During the early part of his retirement he was able to bring all this work together in his second great book, *Animal Locomotion*.

Later in life Gray became increasingly involved in advisory work for national organisations. He was a trustee of the British Museum (1948–60), a member of the Agricultural Research Council (1942–47), President of the Marine Biological Association (1945–55) and President of the British Association in 1959. From 1951–59 he was a member of the Development Commission and at one time Chairman of its Fishery Advisory Committee. For his public work he was made CBE in 1946, and in 1954 he received a Knighthood. He was honoured by many universities, by Aberdeen and Edinburgh with the L1 D., by Durham, Manchester and Wales with the D.Sc.

Gray's influence upon the development of zoology was greater even than his direct contribution to it. He was foremost among a band of rising young biologists who in the years immediately after the First World War led the subject into new paths and established Experimental Zoology in the face of traditionalist opposition. He continued to foster this movement through the Society for Experimental Biology, at whose meetings he was regularly to be seen, and through the Journal of Experimental Biology which he edited from 1925 to 1954. His influence was, naturally, nowhere stronger than in his own university. During his tenure of the Chair the teaching of Zoology was transformed—but gradually, for he was emphatically opposed to unnecessary disturbance and dislocation. This was a time when the director of research and his team of research students were beginning to make their appearance on the university scene. Gray would have none of this. He looked upon research students as independent research workers in their own right; as long as they were swimming he left them alone, if ever they got into serious

difficulty they were dramatically rescued. Gray believed passionately that the mainspring of a successful university department was the activity of the staff in research. What line of research they chose mattered nothing provided that it was scientifically sound and pursued with enthusiasm; for, he argued, this enthusiasm would rub off on the undergraduates and, far more than mere knowledge imparted to them, would bring out their best qualities. One has but to look at the list of zoologists who were at one time research students in Gray's department and then went off to become Heads of Departments themselves to see how well the system worked.

As Head of Department himself he was always approachable, always ready to listen to someone in trouble, always ready to step in where he could be of help. There was also a rough side to his character. He was slow to wrath, but it was always a grave matter when he was aroused. Yet he was scrupulous to keep his personal feelings to himself, and they were never allowed to influence his official policy or decisions. In private life, though he had many very close friends, his happiest hours were spent on summer holidays in Scotland with his family.

**J. A. Ramsay**

**Joseph F. Foster**, Professor of Chemistry in Purdue University and former Chairman of the Department, died suddenly on October 7, 1975 at the age of 57. He was distinguished for his research on the physical chemistry of macromolecules, especially on starch and proteins. He was probably the foremost contributor to our knowledge of the serum albumin molecule during the last twenty years. He demonstrated its reversible expansion in acid solutions, and discovered two Conformational transitions in albumin, one in acid solution (the N $\rightleftharpoons$ F transition) and another in neutral slightly alkaline solution. From this he inferred and demonstrated the microheterogeneity of albumin preparations. He was extending and deepening these important studies up to his untimely death. He will be greatly missed by his colleagues in protein chemistry.

**John T. Edsall**

# announcements

## Awards

In addition to the winners already mentioned of the prizes in Cancer Immunology awarded by the Cancer Institute of New York, were also **Dr Garri Abelev**, N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow, and **Dr Eva Klein**, Karolinska Institute, Stockholm, Sweden.

## Appointments

**Dr Michael Gibbons** has been appointed Professor of Liberal Studies in Science at the University of Manchester.

**Dr W. H. Cockcroft**, at present the G. F. Grant Professor of Pure Mathematics at the University of Hull, is to become Vice-Chancellor of the New University of Ulster in October.

## International meetings

March 1-3, **First World Hydrogen Energy Conference**, Miami Beach, Florida (1st World Hydrogen Energy Conference, Conference Services, University of Miami, PO Box 248005, Coral Gables, Florida 33124).

March 15-19, **Development of Nuclear-based Techniques for the Measurement, Detection and Control of Environmental Pollutants**, Vienna (The IAEA Kärntner Ring 11, PO Box 590, A-1011 Vienna).

March 22-26, **The Management of Radioactive Wastes**, Vienna (The IAEA Kärntner Ring 11, PO Box 590, A-1011 Vienna).

March 29-April 2, **The Exploration of Uranium Ore Deposits**, Mexico City (The IAEA, Kärntner Ring 11, PO Box 590, A-1011 Vienna).

## Miscellaneous

The **Lady Tata Memorial Trust** offers fellowships and scholarships for research on leukemia and allied conditions. The fellowships are tenable for 3 years and the scholarships for 1 year, renewable for 2 more. For further particulars apply to The Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Institute, Fulham Road, London SW3. Applications must be submitted by March 31.

## Reports and publications

Smithsonian Contributions to Zoology No. 204. Taxonomic Indexes to Ostracoda (Suborder Myodocopa) in Skogsborg (1920) and Poulsen (1962, 1965).

## Person to Person

The change in the tensile strength of cellulose on burial in soil is a technique of general application in ecology, but for repeatable results, a high-quality standard test cloth must be available. The Shirley Institute, Manchester has agreed to manufacture, and sell at cost price, such a material if initial demand is sufficient. Anyone who might be interested should contact Dr D. W. H. Walton, British Antarctic Survey, Monks Wood Experimental Station, Abbots Ripton, Huntingdon, Cambs. PE17 2LS

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

By Anne C. Dohen and Louis S. Kornicker. Pp. iii+29. No. 209: Vertical Distribution of Pelagic Cephalopods. By Clyde F. E. Roper and Richard E. Young. Pp. iv+51. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.)

[1811] United States Department of the Interior: Geological Survey Bulletin 1389. Stratigraphic and Structural Relationships of the Brimfield Group in Northeast-Central Connecticut and Adjacent Massachusetts. By John D. Peper, M. H. Pease, Jr., and Victor M. Seiders. Pp. iv+31+3 plates. Water-Supply Paper 2101. Surface Water Supply of the United States 1966-70. Part 1: North Atlantic Slope Basins. Vol. 1: Basins from Maine to Connecticut. Pp. xi+1123. Water-Supply Paper 2143: Quality of Surface Waters of the United States, 1969. Part 3: Ohio River Basin. Pp. x+371. \$2.85. Professional Paper 437-E: Land Subsidence Due to Ground-Water Withdrawal in the Los Banos-Kettleman City Area, California. Part 1: Changes in the Hydrologic Environment Conductive to Subsidence. By William B. Bull and Raymond E. Miller. Pp. iv+71. Professional Paper 725-B: Generalized Geologic Framework of the National Reactor Testing Station, Idaho. By Raymond L. Nace, Paul T. Voegell, James R. Jones, and Morris Deutsch. Edited by Seymour Subitzky. Pp. iv+49+ plate 1. Professional Paper 798-B: Geology and Geochemistry of the Copper Canyon Porphyry Copper Deposit and Surrounding Area, Lander County, Nevada. By Ted G. Theodore and David W. Blake. Pp. v+86+2 plates. (Washington, DC: Government Printing Office, 1975.)

[1811] United States Department of the Interior: Geological Survey Bulletin 1395-B: Revision of the Type Mankomen Formation (Pennsylvanian and Permian), Eagle Creek Area, Eastern Waska Range, Alaska. By D. H. Richter and J. T. Dutro, Jr. Pp. iii+25. Water-Supply Paper 2030: Ground-Water Levels in the United States, 1967-71. North-Central States. Pp. v+114. 80 cents. (Washington, DC: Government Printing Office, 1973 and 1975.)

[1911] Annals of the South African Museum. Vol. 67, Part 6: Pleistocene Molluscs from the West and South Coasts of the Cape Province, South Africa. By R. N. Kilburn and A. J. Tankard. Pp. 183-226. R3.55. Vol. 67, Part 7: The Morphology and Relationships of a Crocodilian, *Orthosuchus stormbergi*, from the Upper Triassic of Lesotho. By Diane S. Nash. Pp. 227-329. R6.30. Vol. 67, Part 8: Archaeocyatha Provenant de Blocs Erratiques des Tillites de Dwyka (Afrique du Sud). Par Françoise Debrenne. Pp. 331-361. R2.90. Vol. 67, Part 9: A New Species of *Meosquilla* (Crustacea, Stomatopoda) from South Africa. By Raymond B. Manning. Pp. 363-366. R1.50. Vol. 67, Part 10: Five Species of *Jacropsis* from the Southern Indian Ocean

(Crustacea, Isopoda, Asellota). By Brian Kensley. Pp. 367-380. R1.85. Vol. 67, Part 11: A Preliminary Catalogue of Identifiable Fossil Fish Material from Southern Africa. By R. A. Jubbs and B. G. Gardiner. Pp. 381-440. R4.40. (Cape Town: South African Museum, 1975.)

[2011] World Health Organisation. Technical Report Series No. 577. Evaluation of Dependence Liability and Dependence Potential of Drugs—Report of a WHO Scientific Group. Pp. 50. (Geneva: WHO, London: HMSO, 1975.) Sw fr. 7.

[2011] Smithsonian Contributions to Zoology No. 191: Cyclopoid Copepods (Lichomolgidae) Associated with Alcyonaceans in New Caledonia. By Arthur G. Humes. Pp. iii+27. (Washington, DC: Smithsonian Institution Press, 1975. For sale by US Government Printing Office.)

[2111] World Health Organisation. Technical Report Series, No. 575: Advances in Methods of Fertility Regulation—Report of a WHO Scientific Group. Pp. 45. (Geneva: WHO, London: HMSO, 1975.) Sw fr. 6.

[2411] United States Department of the Interior: Geological Survey. Professional Paper 433-0: Distribution of Radionuclides in the Columbia River Streambed, Hanford Reservation to Longview, Washington. By W. L. Hauschild, G. R. Dempster, Jr., and H. H. Stevens, Jr. Pp. iv+35. Professional Paper 502-C: Solute Balance at Abert and Summer Lakes, South-Central Oregon. By A. S. Van Denburgh. Pp. iv+29+1 plate. Professional Paper 813-B: Summary Appraisals of the Nation's Ground-Water Resources—Upper Mississippi Region. By R. M. Bloyd, Jr. Pp. iii+22. Professional Paper 836: Stratigraphic Distribution and Zonation of Jurassic (Callovian) Ammonites in Southern Alaska. By Ralph W. Imlay. Pp. iii+28+6 plates. Professional Paper 864-A: A Review and Interpretation of the Geologic Setting of the Watchung Basalt Flows, New Jersey. By George T. Faust. Pp. iii+42. (Washington, DC: Government Printing Office, 1975.)

[2411] World Health Organisation. Technical Report Series, No. 576: Evaluation of Certain Food Additives—Some Food Colours, Thickening Agents, Smoke Condensates, and Certain Other Substances. (Nineteenth Report of the Joint FAO/WHO Expert Committee on Food Additives.) Pp. 23. Sw fr. 5. No. 579: Developments in Malaria Immunology—Report of a WHO Scientific Group. Pp. 68. Sw fr. 7. (Geneva: WHO, London: HMSO, 1975.)

[2711] United States Department of the Interior: Geological Survey Bulletin 1382-C: Argentinian Cryptomelane and Bromargyrite in Volcanic Rocks near Silver Cliff, Colorado. By Fred A. Hildebrand and Elwin L. Mosser. Pp. iii+23. (Washington, DC: Government Printing Office, 1974.) 40 cents.

[2711] United States Department of the Interior: Geological Survey. Water-Supply Paper 2007—Geologic and Hydrologic Features of Indian Wells Valley, California. By L. C. Dutcher and W. R. Moyle, Jr. Pp. iv+30+6 plates. (Washington, DC: US Government Printing Office, 1973.) \$3.75.

[2811] Nederlandse Vereniging voor weer- en Sterrenkunde. Observations of Variable Stars, January/June 1975. (Report No. 28.) Pp. 10. (Groningen, Netherlands: Kapteyn Astronomical Laboratory, 1975.)

[112] OECD Halden Reactor Project—Fifteenth Annual Report. Pp. 103. (Paris: OECD, Nuclear Energy Agency, 1975.)

[112] Republic of Cyprus. Ministry of Agriculture and Natural Resources. Annual Report of the Geological Survey Department for the year 1974. By Y. Hadjistavrinou. Pp. 45. (Nicosia: Geological Survey Department, 1975.)

[112] CERN. European Organization for Nuclear Research. CERN-75-13: AGS—The ISR Computer Program for Synchrotron Design, Orbit Analysis and Insertion Matching. By E. Keil, Y. Marti, B. W. Montague and A. Sudboe. Pp. vi+57. (Geneva: CERN, 1975.)

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[212] Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Rapport Annuel, Exercice 1974. Pp. 507. (Bruxelles: IRISA, 1975.)

[212] CERN—European Organization for Nuclear Research. CERN 75-14: Monte Carlo Calculations of the Neutron Transmission Through the Access Ways of the CERN Super Proton Synchrotron. By H. G. Vogt. Pp. v+37. (Geneva: CERN, 1975.)

[412] New Zealand Department of Health. National Radiation Laboratory Environmental Radioactivity—Annual Report for 1974. Pp. 38. (Christchurch, NZ: National Radiation Laboratory, 1975.)

[412] United States Department of the Interior: Geological Survey. Professional Paper 773: Geologic Structures in the Gulf of Mexico Basin. By Louis E. Garrison and Ray G. Martin. Pp. iv+85+1 plate. (Washington, DC: US Government Printing Office, 1973.) \$1.75.

nature

February 12, 1976

## The Shackleton incident could profit international law

THE incident at sea last week, in which the British research ship Shackleton had to make rapidly for port in the Falkland Islands after a warning shot had been fired across its bows by the Argentine destroyer *Almirante Storni*, must not be seen simply as an isolated event connected with a territorial dispute and a trigger-happy captain. In recent years the freedom of marine scientists to do pretty well what they wanted even within sight of foreign territory has been rapidly eroded, and there is no sign that when the Law of the Sea conferences have run their course life will be much easier for those who need to come fairly close to foreign coastlines. The point which should be abundantly clear is that Argentina is prepared (not for the first time) to take strong action against infringements of what she regards as her own economic zone by research vessels. She, along with many other countries, has sought to have the power to regulate scientific research within that zone.

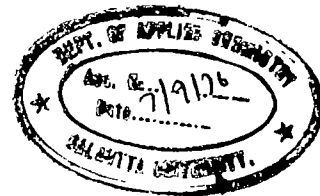
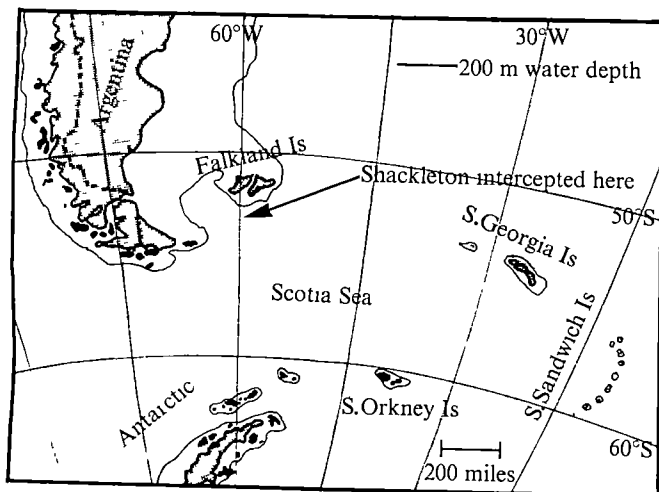
The research conducted from Shackleton used conventional geophysical techniques: magnetometry, gravimetry and refraction and reflection seismology. Most of it was being carried out in deep water in the Scotia Sea, and the programme, under the supervision of Birmingham University, has been running for many years. The purpose of the work is to attempt to put the unique area off South America into a plate tectonic context.

Those countries, Britain included, which have attempted

to produce guidelines for international discussion on what is pure and what is applied research would almost certainly rate Shackleton's operations as 'pure'. But it is not as simple as that. Theories of the regional evolution of the Earth's crust have a lot in them for the economic geologist and thus for the prospecting industry; a clear picture of past geological history will not point a finger at exactly where hydrocarbons will be found, but it will provide some helpful signposts. Reasonably, scientists may protest that their work is the pursuit of knowledge for its own sake, that it is not supported by commercial interests, and that their results will be available internationally. But equally reasonably, countries with inadequately developed technology and expertise to capitalise on this information may feel themselves consistently outmanoeuvred when it comes to negotiating exploration rights. Their only defence then may be to demand some sort of technical assistance in exchange for access.

In a month's time the Law of the Sea Conference will resume in New York, and among other things will attempt to come to terms with a 'single negotiating text' including draft articles on marine scientific research. It is likely that the coastal state in whose economic zone another nation wishes to perform research will be called upon to decide whether the research is 'pure' or 'applied' and, in the former case, presumably to grant permission without too many strings attached. An appeal procedure to international experts is proposed if agreement cannot be reached. This seems a much better approach than any attempt by the United Nations to produce a universally acceptable list of topics to be regarded as pure.

It will be a pity if the Shackleton affair cannot be viewed at the conference as more than a territorial dispute. For, squabbles apart, it provides a rather good concrete example of the sort of research operation that is going to give rise to international disagreement; yet there is no reason why intelligent give and take on technology transfer and technical advice should not resolve such disputes. If this incident could be freed from its accretions of diplomatic conflict and considered simply as an ideal case for the Law of the Sea Conference to consider when legislating, some good could come of it. But it will need a certain detachment from the British delegation. □





## Looking at the job first

*In Nature last November, Cyril Cooper of the Institution of Professional Civil Servants highlighted the discontent among UK Civil Service scientists, and argued that it stemmed largely from differences they experience in salary and career progression compared with graduate administrators. David Budworth of the Confederation of British Industry replies.*

THE NEWS that the Expenditure Committee is to examine the progress made in implementing the 1968 Fulton Report on the Civil Service is indeed welcome. Although the stimulus has no doubt been the rising public indignation at the advantages in terms of pay, security, and perhaps most conspicuously pensions that civil servants enjoy, the terms of reference of the investigation seem likely to be sufficiently wide to permit the Committee to tackle the problem of civil service scientists, which has been consistently ducked in recent years.

While it may well be good trade union tactics to argue, as Cyril Cooper does (November 20, 1975, page 186) that the scientific civil service is really just an ordinary part of the higher reaches of the service and should be treated as such, this approach flies in the face of economic reality and common sense. It is certainly also in flat contradiction of the Fulton principle of "look at the job first". Regrettably perhaps, but undeniably, the number of jobs available for administrators in the civil service has increased, and with it the security and career prospects of the incumbents, in our developing welfare state in which authority is looked upon to solve all the problems.

Science having conspicuously failed in its overblown claims to solve these problems, the opportunities for scientists have not expanded in a similar way. As David Davies has reminded us (May 22, 1975, pages 293-296), the scientific civil service has been static in numbers for the best part of twenty-five years, and it suffers from considerable internal embarrassments caused by its over-concentration of older scientists. Yet these under-used and probably unhappy people are a considerable national resource of talent and experience. Diverting them to supposedly useful tasks in their present locations is unlikely to do much of value. They must be moved out to

contribute to solving our national problems in the places where it is increasingly being recognised that something effective can be done. industry and the schools.

A Select Committee such as the Expenditure Committee is perhaps the only type of body which can look at these problems relatively free from civil service establishment influence. The Fulton Committee itself brushed aside the suggestions made to it, significantly including some from inside the service itself, that the scientific civil service would benefit from not being a career service; and Lord Rothschild's forthright comments on the immobility of scientific civil servants were safely defused by the terms of reference given to the Bondi task force which was set up to tackle the problem.

It is greatly to the credit of that body and of its chairman, whose personal commitment to mobility has been amply proved by deeds as well as words, that it did manage to rise slightly above its terms of reference and record its view that permanent moves were, in the end, more important than the immensely difficult and therefore marginally effective secondments to which it was nominally confined. Ironically perhaps, the work so far of the interchange unit set up in the Civil Service Department to keep the flame of scientific mobility alive, seems to have proved the critics of civil service privileges right, for the movement into the service has greatly exceeded the movement out.

Of course, as is apparent from Cyril Cooper's article, all pretence that the "fair comparison with outside employment" system for determining pay applies to civil service scientists has been abandoned, following the report of the late Pay Board's Advisory Report No 3 of April 1974. This body again tamely accepted that something closely approximating to parity should exist between specific grades in the

administration and scientific groups of the service.

The reforms in the scientific civil service which are needed to effect a long-term cure are fairly clear in outline, and have been put forward so often that there is no need to repeat them here; but what of the short and medium term? We cannot afford to wait another 30 years or so, even assuming that the necessary changes are made, for the situation to right itself. Something must be done before that, and preferably soon. The former Department of Trade and Industry admitted in a burst of frankness in 1972 that it wanted to reduce the burden that its laboratories placed on public expenditure, but its attempts to do so by selling contract research cannot have made much impact. The unproductive PSOs mentioned by David Davies can hardly be declared redundant, particularly with unemployment at its present levels; but could not some way be found of getting them out to somewhere where their undoubted abilities might be put to use? From all accounts, good quality scientists and mathematicians are still not coming forward for teaching posts, and even when they do it seems that local authorities cannot afford to pay them. Is it totally beyond the wit of what is supposed to be the best administrative civil service in the world to devise some methods of utilising those on the public payroll to do the jobs which need to be done?

Again, if the Government is serious in its commitment to putting the needs of manufacturing industry above almost all other calls on its resources, there will be a need to move more brain power, and to move it pretty quickly, to where it can be commercially effective. Some scientists in government laboratories have proved themselves to be effective in marketing their services. Perhaps they could be equally useful in marketing the mechanical engineering and other products by which we live. Or will the real gap in our country, that between those who live on taxpayers' money by the skilled deployment of political argument, and those who live by exploiting their entrepreneurial abilities in some kind of market, be allowed to get even worse, so that we all eventually starve?

# "Genetics" and how we made it

Steven Rose, Professor of Biology at the Open University, looks at the origins and purpose of the OU's new genetics course



SINCE the beginning of this month some 900 Open University students have been settling down for their standard 12 hours study a fortnight to follow a new second level Genetics Course—a set of 16 sequential study Units ranging in context from classical genetics by way of molecular and cytological genetics to population and human genetics, each with an accompanying TV and radio programme and a series of "home experiments". What makes the Genetics Course different from all the rest of the OU science teaching, however, is that it is the result of almost 3 years of collaboration between the Biology Department of the OU and the Department of Genetics at Birmingham, the Unit of Genetics at Hull, the School of Biology at Sussex and a member of the biology staff at the University of York. Teaching staff from the four collaborating universities were released from most of their other teaching duties in order to work on the production of this Course—a participation made possible by a grant from the Nuffield Foundation, which has an interest both in promoting inter-university teaching projects and in the development of the type of structured teaching that the OU has pioneered at University level. But the involvement was not just that of individuals, for each participant had the support of his department and access to its own particular area of genetic and teaching expertise. The result has been a novel Course that can be used both by the OU and in many other teaching institutions.

There were several reasons for choosing genetics for this experiment. The modern synthesis of genetics underlies most areas of biology. It is a subject that, throughout its history, and to the present day, has been interwoven with social and philosophical controversy concerning its interpretation and implications. It is very well suited for a problem-oriented, student-active teaching approach. And finally, a prosaic but important point is that not even all universities, and certainly rather few polytechnics and colleges, have biology schools large enough to be able to teach Genetics across the full range, from the molecular to the population and human levels. Hence the brief of the Joint Universities Genetics Course Team has been to prepare a Course that will eventually be able to be used in whole or in part by a very wide range of institutions even when they do not have a full range of genetic

skills represented amongst their teaching staff.

Working as an inter-university team has had both its pleasures and its perils. We came into the venture with differing views, based on our differing experiences, not merely on *what* should be taught but on *how* it should be taught. University teachers tend to be individualists, but, meeting practically monthly since 1973, together with the BBC staff, educational technologists, technicians and researchers who comprise the full Course Team, has welded us into a collective teaching group. In such a team, every assertion about what should be included and how it should be approached has to meet the challenge and critical assessment of our colleagues. We have been able to seek the advice of expert assessors outside the team to comment on various sections of the Course during its development and to call on a panel of students to assess the drafts of the Units for their success as teaching documents, so as to point to areas of potential obscurity or theoretical or factual overload. It has been particularly instructive and we believe useful to have had, for instance, biometricians confronted with developmental geneticists and being forced to convince each other—and the rest of us—of the case for including their respective approaches in a Course in which every minute of the students' time is precious and academic pretentiousness or hobbyhorses cannot intrude. One result, incidentally, has been a considerable rethinking of the amount of mathematics that is essential to an understanding of biometry at this level and a series of experiments in ways of presenting it which we have been able to test with a number of volunteer students.

## The Course itself

The Course itself, seen as a linear sequence (although each of its Units may be used separately) begins by asking: What is genetics? What sorts of questions do geneticists ask and how do they obtain the answers? Not surprisingly it goes back to Mendel, and includes a reprint of a translation of his original (1865) paper at this point. There follow four Units on chromosomes and genes; recombination; linkage and maps; and chromosome organization and changes. The next three Units cover molecular genetics, cytoplasmic inheritance and developmental genetics and there are five Units on the analysis of populations, biometry, plant

and animal breeding and evolutionary and ecological genetics. The final two Units focus on human genetics, and confront some of the major currently controversial issues in human genetics (for instance the question of whether the biometrical approach has anything to contribute to the analysis of group differences in IQ score, the XYY programme and the prospect of genetic engineering). The main teaching sequence is accompanied by a parallel Unit which sets present-day genetic theories into the context of their history and social relations (not forgetting past controversies like that around eugenics and social Darwinism). There is also a specially prepared statistics Unit. All the texts are designed to be self-instructional, with opportunities for the students to assess their own progress by tackling problems *en route* through the Units (OU students will also have a series of programmed tutorials through the year).

Integral to the teaching material are the video and audio tapes, which will be transmitted fortnightly by the BBC over the year, beginning this month. The TV includes both studio animations and experimental demonstrations and also film material involving many leading geneticists, both in this country and abroad, in their own labs or in the field. The radio has a varied role, from radio-vision (for example of fine-structure genetic mapping and the derivation of biometric equations) through to historical programmes on the development of phage genetics and the Lysenko episode. Among those taking part in radio or TV programmes (apart from members of the Course Team itself) are Bradshaw, Cain, Crick, Delbrück, Edgar, Lewontin, Levins, Luria, Sager, Sonneborn, Stent, Stern, Suzuki and Watson.

Finally, for OU students there is a "package" of experiments to be done at home which include genetic studies on tomato seedlings, on micro-organisms and *Drosophila*, as well as a set of observations to be made on humans which will be sent back to be analysed centrally for their genetical implications.

For OU students taking the Course this year, it will be just one component of their degree programme. But at the

same time they will be participating in the final stages of a substantial experiment. Throughout the year we will be monitoring their progress, and that of

students at some conventional universities and colleges who are also using the Course or parts of it. For only this will tell us how close we are to our

ultimate objective—a first rate and comprehensive second level genetics teaching Course which can be widely used in Britain and abroad. □

## USSR

# Environmental protection under state socialism

*In the Soviet Union environmental issues have surfaced in recent years as just one consequence of the country's rapid development. The problems seem as intractable there as anywhere else. Vera Rich reports.*

ECOLOGICAL problems seem to be as much a concern of the Soviet Union as they are of other industrialised countries. The prospectus for the Five-Year Plan lays considerable stress upon conservation and environmental protection, and the science and technology section includes among its major aims: "to study the scientific principles of the use and conservation of soils, mineral resources, the plant and animal worlds, the air, and water basins. To expand complex investigations of the world's oceans. To effect the further development of methods of forecasting the weather and natural disasters".

Commenting on this programme in the English-language *Moscow News* (No. 1, 1976), Academician Inokentii Gerasimov implies that the problem of conservation, although a real one, arising from rapid urbanisation and industrialisation, is nevertheless qualitatively different from that in capitalist countries: in the Soviet Union, he argues, "there are no social reasons for the irrational utilisation of natural wealth. Environmental protection under socialism, with its planned economy and no privately owned natural resources, becomes an important task of the state. Hence, environmental protection in our country is part of the current and long-term economic plans".

Taken at its face value, Gerasimov's comment would imply that, in the Soviet Union, any ecological hazard would be of a short-term nature only: the result of a temporary imbalance between one part of the Plan and another. Such short-term imbalances do occur, and at a fairly low level of the administrative hierarchy may become the subject of criticism in the media. Last November, for example, Minsk Radio noted that reafforestation (to replace planned felling) in Byelorussia was falling behind schedule,

while in the Brest, Minsk and Grodno Oblasts, excessive felling had taken place and the authorities concerned had failed to take the required "essential measures" against fire and timber-poaching. "Anti-social elements"—fish-stealers, litter-droppers, and others who have not yet learned the norms of behaviour proper to a socialist society—present another possible environmental threat. They too are the subject of exhortation in the media, particularly at the local level; on occasion, the state-sponsored Society of the Friends of Nature has been called upon to organise vigilantes to deal with a particular outbreak of "hooliganism".

These, one might conclude from Gerasimov, are the main dangers to the Soviet environment—fairly local and minor disturbances, far down the administrative and social ladder. In fact, the problem is somewhat more complex. The Soviet authorities have in recent years become increasingly aware that their natural resources, though vast, do not constitute the infinity promised to Peter the Great by his geographers. At the Twenty-Fourth Party Congress in 1971, Mr Brezhnev stressed this new approach: "As we take steps to accelerate scientific and technological progress," he said, "we must ensure that this is combined with the rational use of natural resources and should not cause dangerous pollution of air or water, nor exhaust the soil".

This marks a fairly new approach. The early years of the Soviet Union were marked by rapid industrialisation. On posters and cartoons, the smoking factory chimney became the standard symbol for progress. In accordance with Lenin's equation (Communism = Socialism + Electrification), rivers were dammed and spillways built with little, if any concern for the ecological consequences. The writings of Marx dwell mainly on the urban proletariat and barely touch on conservation; and from Lenin's work isolated quotations can be extricated to serve as slogans. Concern was directed more towards utilising than conserving natural resources, of the major theoreticians, only Engels showed concern with the long-term outcome of remodelling the environment. For many reasons, per-

haps ideological as well as practical, the relevant passages seemed to make little impression on the planners. To turn rivers aside from their courses, to plant orchards where there once were deserts, to carpet the barren steppe with horizon-to-horizon grain-fields—such achievements, as well as bringing immediate economic benefits, would demonstrate at home and abroad the intrinsic superiority of the Soviet system of planning.

In the 1920s, of course, in spite of industrial expansion during the last decades of Tsarism, the vast majority of the Soviet Union was still in the state of a country emerging from feudalism, and a system of tied serfdom remained within living memory. Thus, after some elementary laws were passed—the nationalisation of land, the prohibition of fish poaching, the establishment of certain national parks and health resorts—little attention was given to ecological problems: the drive to take the country into the twentieth century outweighed all other considerations. Only two major pieces of conservation legislation were passed under Stalin. These dealt with the establishment of "shelter belts" of trees to prevent erosion (1948) and the control of air pollution (1949), and the first remained something of a dead letter.

Only during the late 1950s, under Khrushchev's policy of opening up undeveloped areas, were a large number of laws passed on conservation and the environment, reflecting a growing awareness of the need for a coherent policy in this field. In many cases, the laws passed at that time set extremely high standards, as though they were intended as "socialist ideals" rather than practical policies: in certain cases virtually the same law was promulgated several times, suggesting considerable difficulties in implementation. Nevertheless, the laws were published in the press and became common knowledge. In the atmosphere of "the thaw", concerned individuals began to voice protests against the most flagrant infringements either officially, or, later, through the *samizdat* literature. Furthermore, the growing international concern with conservation impelled the Soviet authorities, if only for prestige reasons, to show that the homeland of socialism was not lagging behind in its concern about a universally pressing problem. It is now clear that many of the "developers" were, by present standards, carried too far by their zeal. Many recent Soviet "achievements" in



conservation are, in fact, motivated by the need to clean up the mess of a former generation.

### The case of Lake Baikal

A classic example of this is the case of Lake Baikal. This lake, the deepest in the world, has long been famous for its pure water and its extensive flora and fauna, with 708 unique types of living organisms, including freshwater seals. In *Moscow News* Gerasimov announced proudly, "It can be definitely said now that Lake Baikal will not die of pollution, as have many other lakes in the world". Under the Tsars, virtually the sole contact between Lake Baikal and the world of industry was the Trans-Siberian railway which skirted part of its southern shore. Inevitably, perhaps, the forests of the Baikal region came to be utilised, and the tributaries of the lake used for rafting timber prior to its further shipment down the Angara, Baikal's sole outlet. But the construction of cellulose and pulp mills in the unique habitat of Lake Baikal seemed odd in a system claiming to use natural resources "rationally" for the good of present and future generations.

The plans for the mills were approved in 1957, but it was not until 1962 that the first authoritative protests began with an article in *Komsomolskaya Pravda* by the Director of the Limnological Institute of the Siberian Branch of the Soviet Academy of Sciences. Over the following decade, numerous similar articles were published, notably in *Literaturnaya Gazeta* and *Priroda*; Gerasimov himself was prominent in the campaign. Since campaigns of this nature in the State press (as opposed to the *samizdat* network) can only take place with tacit approval from Government bodies involved, there was either a high-level interdepartmental dispute over Lake Baikal or at least an effort to show official concern.

The indications are that there was opposition from the Academy of Sciences, the Geographical Society of the USSR, and the Expert Commission for the Coordination of Scientific Research. Nevertheless, under the auspices of the Ministry for Timber Production, the mills were built and began operation, discharging effluent into the lake. Even when effluent treatment installations were fully operational, the water discharged was yellowish and barely potable, and by no means comparable in quality with the original lake water. The only sound "economic" reason ever offered for the siting of the mills was the exceptional purity of the Baikal water—a purity needed for the production of certain high-quality products. As early as 1965, however, it was observed that water from the

effluent outfall was being carried back by the current to the "pure" water inlet of the mills (*Priroda*, No. 11, 1965). Consequently it was necessary to process the intake water before use. This demanded the installation of expensive pre-treatment equipment the authorities had hoped to avoid by using Baikal water in the first place.

During the last five-year plan, considerable attention was devoted to the problem of the Baikal habitat. New legislation with Party backing was introduced in September 1971, and a special "emergency charter" was approved by the Ministry of Land Reclamation and Water Economy in November 1974. Timber-felling was forbidden within a radius of 50 km of the lake, and the tributaries of Baikal have been cleared of the sunken timber which might have absorbed much of the oxygen from the water and covered fish-breeding grounds; "very costly" outfall treatment plant has been installed at the cellulose mills, and white-fish hatcheries have been introduced to replenish depleted stocks.

Lake Baikal has always been of keen interest to ecologists throughout the world, but more recently it has attracted increased attention because of the construction of the Baikal-Amur Railway. Ecological restrictions relating to this at times verge on the absurd (workers must not spray mosquitoes with insecticide) or over-zealous (devastated areas from natural forest fires on the route are to be planted with seedlings, rather than left to regenerate naturally). But if all the recommendations are carried out, the future of Baikal is certainly assured, and the lake and its environs could well become an ecological show-place. The decision to admit American scientists to observe and participate in research on the lake suggests that already the worst hazards have been dealt with, which is gratifying. But the general question remains whether state ownership of resources entails their best use and protection.



Freshwater seal from Lake Baikal

## CANADA

SINCE the beginning of the year the outlook for Canadian science and technology for 1976 has not seemed a particularly optimistic one. The Ministry of State for Science and Technology, contrary to some predictions, did manage to survive the federal government's anti-inflation programme which killed off a number of other federal initiatives such as Information Canada, the Company of Young Canadians and Opportunities for Youth, and reduced others, such as the Local Initiatives Program. But the programme still means less money for science and technology generally.

Specifically, the Industrial Research and Development Incentives Act, which has provided between \$20 million and \$30 million a year for industrial research, will be repealed; reductions will be made in the Programme for Advancement of Industrial Technology and the Defence Industry Productivity Programme; and medical and other scientific research grants will be frozen.

The cuts were made in an attempt to prove to Canadians that the federal government was serious about fighting inflation, and that it intended to set an example. But since then the Prime Minister has gone further, telling the Canadian people that the anti-inflation measures are in fact attempts to control an economy that proved itself unable to work as a free market system—a remark that produced angry responses, including a call for an election by a former Progressive Conservative Cabinet minister.

Altogether, the government said it would cut \$1,500 million from its future spending plans. The loss to scientific research funds was estimated at \$14.8 million and to industrial incentives at \$8 million. These are losses that the scientific community mostly regards as insupportable, in the light of the federal government's recent policies and the impact of inflation.

In a letter to the editor of the *Toronto Globe and Mail*, John Polanyi, Professor of Chemistry at the University of Toronto, pointed out that the total funds available to the National Research Council (NRC), the chief funding body for fundamental research in Canada, will have increased at an average rate of only 2.5% a year from 1969 to 1977, while the cost of doing research during the same period had increased by 100%.

The NRC grants committees, on which he has served, "are quite unable to keep existing research projects of high promise moving ahead, while at the same time giving a genuine opportunity to the scientists of tomorrow to prove their mettle". And, he went on,



"our neglect of science is something that sets us clearly apart from countries with which we might reasonably compare ourselves. In the United States, in France, in Germany, even in beleaguered Britain, the support of basic science has roughly kept pace with inflation. Only in Canada has inflation been used, year after year, as a device for diminishing the nation's investment in this fundamental activity."

In a letter to the same newspaper, Dr Gordon Forstner said that in medical research "somewhere between 150 and 300 technicians will lose their jobs, and a programme which has been deflated steadily during the last five years will be shattered."

● In such a climate, it was with mixed feelings that some Canadians heard that the heavy water plant at Glace Bay, Nova Scotia, was starting up and would shortly produce heavy water not only for nuclear reactors in Ontario and Quebec but also for those in Britain, Argentina and South Korea. Although heavy water is vital to Canada's CANDU reactors (in which it acts as coolant and moderator), the Glace Bay plant has been one of the most disastrous undertakings of an otherwise successful nuclear programme.

In 1964, the province of Nova Scotia brought in a US nuclear scientist, Jerome Spevack, to design the Glace Bay plant. There were difficulties from the outset. Completion was delayed from 1966 to 1967, then again to 1969. Technical difficulties occurred as a result of using salt water from the nearby Atlantic Ocean in the process, instead of fresh water. Finally, in 1970, inspectors discovered that the salt water had corroded the pipes, and a \$30 million repair was needed.

By that time, Spevack's company, Deuterium of Canada Ltd, had spent \$100 million on the plant, all of it public money, because Nova Scotia was the major shareholder and provided the finance. The province had bought out Spevack's interest for \$3 million in 1966, and taken full control in 1969. In 1971, the federal government provided the funds for the plant's purchase by Atomic Energy of Canada Ltd (AECL), and spent another \$130 million on what has been almost a complete reconstruction.

Eventually, the plant is expected to become self-sustaining and make enough money to pay back AECL's investment. The Nova Scotia government is to get the plant back after AECL has recouped its investment, but the province plans them to sell it back once more to AECL—finally washing its hands, as it were, of the whole affair.

**David Spurgeon**

Ottawa

USA

## Alternative technologies urged in pesticide report

*Carried to excess, the best things may do more harm than good. Colin Norman reports from Washington on a study of pesticides.*

A COMMITTEE of the National Academy of Sciences has warned, in a mammoth study published last week, that unless alternatives to conventional chemical pesticides are swiftly developed and adopted, agricultural production in the United States could soon begin to suffer. The committee bases that conclusion on the fact that several potent products have been either severely restricted or banned entirely because of environmental and health hazards, and also the fact that several pests are developing resistance to the poisons which are sprayed on them in copious quantities each year.

The study, which took more than three years to complete and which runs to five massive volumes, is an attempt to assess the current state of the art in pest control and to pinpoint some of the problems which lie ahead. In the course of its analysis, the committee has questioned the costs of some government regulations, criticised current pest control practices, cast doubt on the value of some of the Department of Agriculture's most ambitious and costly efforts to eliminate particular insect species and, by implication, criticised the federal government's agricultural research efforts.

The basic theme running through the huge tome is that although chemical pesticides have served agriculture—and, for that matter public health pro-

grammes—very well, the problems of declining effectiveness "warrant substantial expansion of present efforts to promote alternative technologies, including integrated pest management". The chairman of the committee, Dr Donald Kennedy, professor of physiology and zoology at Stanford, said last week, for example, that genetic resistance to toxic pesticides is growing at an "alarming rate", and he suggested that unless effective alternatives are developed, some agricultural land could conceivably be taken out of production.

As for specific alternatives, the committee notes that so-called 'third generation' compounds, which affect hormonal development or reproductive processes in insects, have some desirable qualities, but it suggests that "there is reason to be pessimistic about the prospects for controlling major crop pests with these compounds." One potential problem with third generation agents is that resistance is likely to develop to them, the committee states.

Insect control by pathogens, such as baculoviruses, is especially promising, but the committee cautions that large-scale use of such agents will require improved methods for culturing insect host tissues. Moreover, the development of new agents will require some advances in basic research. The use of genetic techniques, such as breeding resistance to pests into crop plants, and introducing genetically modified pests into the environment, are also promising, but again the committee



Crop-dusting: must an alternative be found? (Photo: Popperfoto)

cautions that there are potential problems facing widespread application of some of the techniques. The use of sterile male insects to reduce reproduction in a target population, for example, is effective only when the population has been reduced by other means. Moreover, since potential markets for sterile male insects are likely to remain small, there is little commercial incentive for private firms to get into the business of producing them.

Thus, the general message is that although various alternatives are promising, there is no magic 'insect zapper', as Kennedy put it, to replace chemical poisons in the near future. "The task of pest control over the next 10 years will almost certainly become larger rather than smaller", the committee concludes. It therefore recommends that research efforts be stepped up, and in particular that basic research on organisms and ecosystems be given more attention by various government agencies.

As for efforts designed to eradicate individual pest species entirely, the committee warns that such schemes have limited chances of success and frequently turn out to be inordinately expensive. In particular, the committee recommends against a programme,

strongly urged by some scientists in the US Department of Agriculture, which is designed to eradicate the boll weevil from the cotton fields of the southern United States. The scheme, which would employ a variety of pest control techniques, would cost about \$1,000 million. Although extensive trials conducted in 1972 indicate that the programme would have a good chance of success, the committee suggests that there is considerable doubt that the boll weevil could be eradicated entirely. The committee cautions that if the programme fails, it may endanger public confidence in the alternative methods of pest control which would be used.

One of the problems which the committee encountered in its research was an appalling scarcity of data on pesticide use in the United States. "The pest control enterprise places a billion pounds of toxic materials into the environment each year", the committee states, "but it is considered 'normal' for us to have only the vaguest idea of how much each compound was used where, and then only after a decade lag". It therefore urges that much more effort be put into monitoring pesticide use and that chemical companies be required to report their production and sales figures to the federal government. □

### OSETP progress

A longstanding desire of the scientific community in the United States came a step closer to reality last week when the Senate unanimously approved a bill to re-establish a science policy office in the White House. The bill is similar in many respects to a version passed by the House last November, but there are a few important differences which must be ironed out before the measure is sent to President Ford for his signature. The Senate bill would establish an Office of Science, Engineering, and Technology Policy in the White House, headed by a Director who would also be the President's Science Adviser. Unlike the House bill, the Senate version specifies that the director of OSETP would sit on the powerful Domestic Council and be a statutory adviser to the National Security Council. The Senate version would also set up a programme designed to strengthen science policy arrangements at the state level, and to provide grants to states for the application of technology to various pressing domestic problems. It is now expected that the bill will be ready for Mr Ford's signature by the end of March.

## Triple resignation

THE embattled nuclear power industry in the United States suffered a potentially severe political setback last week when three senior engineers in the reactor division of the General Electric Company (GE) quit their jobs and announced that they will campaign for anti-nuclear groups. They said in their letters of resignation that they have become so concerned about questions of reactor safety, proliferation of nuclear weapons, and radioactive waste disposal that they can no longer work for the industry and keep their doubts to themselves.

Each had spent his entire professional career working for GE and, until they quit on February 2, all three were employed at the company's facility in San Jose, California. Although they have raised no new issues, their espousal of the anti-nuclear cause will clearly have substantial impact on public concern about the hazards associated with nuclear power.

That impact is likely to be most keenly felt in California where, on June 6, voters will determine the future for nuclear power in that state. All three have announced that they will campaign heavily in support of a

proposition, to be put to a state-wide vote during the California Presidential primary election, which would almost certainly halt construction of nuclear power plants in California and eventually lead to shut down of existing plants there.

News of the resignations prompted swift reactions in Washington. Congressmen and Senators who have previously expressed concern about nuclear power issued statements publicising the development; Senator John O. Pastore, the chairman of the powerful Joint Committee on Atomic Energy, has tentatively scheduled a committee hearing on February 18 at which the three engineers will testify; and the head of the Nuclear Regulatory Commission, William A. Anders, met with them last week to hear their concerns.

At a press conference called by the Union of Concerned Scientists, an outspoken anti-nuclear organisation, the three engineers said last week that no single event prompted their departure from the world's largest manufacturer of nuclear equipment. All three said that their concerns have been growing for some time, and they felt that the nuclear industry has been seriously downplaying the risks

associated with nuclear technology.

The three engineers are Dale G. Bridenbaugh, formerly Manager of Performance Evaluation and Improvement, who has headed a special project to assess the adequacy of the primary containment vessels of GE's nuclear reactors; Gregory C. Minor, former Manager of Advanced Control and Instrumentation, who has been responsible for design of safety systems and control room instruments, and Richard B. Hubbard, former Manager of Quality Assurance, who has been responsible for ensuring that GE's reactor equipment meets federal quality standards.

Though all three said they reached their decisions to leave the nuclear industry independently, Minor's letter of resignation probably sums up their feelings with the statement that he is "convinced that the reactors, the nuclear fuel cycle, and waste storage systems are not safe. We cannot prevent major accidents or acts of sabotage. I fear that continued nuclear proliferation will quickly consume the limited uranium supply and force us into a plutonium-based fuel economy with even greater dangers of genetic damage and terrorist or weapons activity".



## ISRAEL

# Harnessing science and industry

*As the need to coordinate the work of science and industry even more closely becomes increasingly apparent to the recession-bound developed countries, Nechemia Meyers reports from Israel on the debate taking place there.*

"PLANS for increasing the scientific potential of Israeli industry were ready four or five years ago, but no one seemed to be in a hurry to implement them. Now, under the pressure of our worsening economic situation, people in science, government and industry are beginning to think and act much more realistically". So says Dr Eliezer Tal, Director of Israel's National Council for Research and Development.

This new realism comes none too soon, for it will be required if solutions are to be found to the problems Israel now faces. First, Israeli industry must turn out more goods that can compete on the world market and, as Dr Tal points out, these will not come from the labour-intensive enterprises that were opened with such fanfare in the 1950s and 1960s. "In view of Israel's European-level wage structure," Tal argues, "she must concentrate on the development of high-technology products equivalent in price and—more important—in quality with those coming from the USA, western Europe and Japan".

Another problem intimately linked with this is the one of finding jobs for science graduates. The country's seven institutions of higher learning are fully staffed, and with budgets for higher education on the decline—they went down 30% in real terms during the past two years and will drop still further this year—some lecturers may even be dismissed. Only a more scientifically oriented industrial sector could, at least theoretically, absorb thousands of 'surplus' science graduates, products of local universities and immigration alike.

A controversial approach to achieving both these goals was recently offered by Richard S. Rosenbloom, Professor of Business Administration at Harvard and, of late, Visiting Professor at the Hebrew University of Jerusalem. The primary problem, as he sees it, is not that Israel spends too little on research and development. On the contrary, the country devotes a larger share of its economic resources to those ends, about 2.4% of its GNP, than any Western nation except the

USA. Even when military research and development is discounted, Israel's expenditure, 1.8% of its GNP, remains greater than the total effort of Belgium and Denmark, and about the same, per capita, as spending on civilian research in Sweden and France, although less than in the USA, the UK and the Netherlands. What disturbs Rosenbloom is that only 14.6% of Israel's civilian research and development is carried out in the business sector, as compared to 50% in Denmark and 80% in Switzerland. And the universities, where most of the other research and development is carried out, are not geared to matching technological opportunity with market needs.

What is the answer? Professor Rosenbloom has a revolutionary suggestion: the creation of an entity he calls "Israel Inc.", which would provide a countrywide framework for the innovative exploitation of technology. Israel Inc., although prepared to consider any area of research and development, would probably concentrate its attention on those where existing achievements indicate that meaningful results are most likely to be achieved, including chemicals, drugs, aircraft, industrial machinery, electronics and instrumentation.

Realising that the idea may sound "strange and impractical", Rosenbloom cites as a possible parallel the experience of General Electric (GE). GE, a highly diversified company which employs 307,000 people (as compared to the 250,000 employed by all industrial companies in Israel), has sales roughly four times as large as those of Israeli industry as a whole, and, with 700 identifiable product lines, covers almost as wide a spectrum as all manufacturing companies here put together. GE might serve as a model, Rosenbloom says, because it is not a single monolithic body, but rather a collection of quasi-independent operating units which are, at the same time, subject to certain strong influences from a central organisation.

The first step towards "Israel Inc." would be the creation, under government auspices, of a National Council for Industrial Technology, which would include the heads of all large science-oriented companies, representatives of some of the smaller companies, academics from pertinent university branches and senior government officials. Sub-groups of the council would deal with such topics as market projections, technological

evaluations, manpower, funding and so on.

Next on the Rosenbloom agenda would be the establishment of an Authority for Industrial Technology, managed by the council and owned jointly by industry and government. The authority would operate its own national research and development laboratory and, at the same time, stimulate contact between research and development establishments in various sectors of the economy. Engineers and scientists employed by the authority would not only work in its laboratory, but also be sent out to other research and development centres and to universities. This system of rotation, Rosenbloom believes, would serve to break down the barriers that now hamper the flow of knowledge into industrial innovation and cut off contact between researchers and the markets in which their knowledge could be exploited.

Dr Tal agrees with Professor Rosenbloom on many points, including the advisability of merging the welter of small companies which now exist into large industrial units where serious research and development becomes possible, and the need to link industrial research with industrial policy. The Israel Government, Dr Tal declares, must decide on specific industries to promote, rather than encouraging investment in a wide range of enterprises. He would, however, shy away from the establishment of something like "Israel Inc.", which "might stifle initiative rather than promote it."

Attitudes inside industry also had to change. All large American firms, Tal points out, have a Vice President for research and development who plays a key role in policy decisions. This is far from being the case in Israel where, for example, it was only a few weeks ago that one of the country's largest companies even appointed a top level adviser on technology. Vice Presidents are still undreamed of.

A Swiss-born chemist, Rene Bloch, who heads a small but rapidly developing company engaged in work on synthetic membranes, has his own suggestion for the promotion of industrially oriented research. "To attract our most brilliant scientific minds to industry, we must give them more money and, particularly, more prestige than their university colleagues." As a symbolic first step, Dr Bloch suggests that the country's major national scientific awards—the Israel Prize and the Rothschild Prize—should henceforth go not to "ivory-tower types whose research pays off in papers", but to applied scientists whose research pays off in dollars. □

## IN BRIEF

**Concorde verdict**

The US Secretary for Transportation's 16-month probationary clearance for Concorde, which immediately inspired the Environmental Defense Fund to apply to the District Court of Appeals for a review of the verdict, is still expected to receive Presidential support, despite hostile Congressional opposition. A decision on access from the airport authorities involved is now awaited: the Port Authority of New York and New Jersey, responsible for Kennedy airport, is reportedly anti-Concorde, but Washington's Dulles Airport is Federal controlled.

**JET set?**

Strong demands for a final decision on the siting of the Joint European Torus (JET) are likely when the EEC council of research ministers meets at the end of the month, and Britain looks like losing not only the battle to have the £70 millions fusion project sited at

Culham but possibly the 40 scientists engaged on preliminary work there as well—with Britain apparently isolated in its opposition to the EEC Commission's recommendation that the project should go to Ispra in Italy, the United States is rumoured to have started moves to collect Culham talent for its own fusion research programme.

**AGRs start**

Years behind schedule, two of Britain's highly expensive 1,250 MW advanced gas-cooled reactors (AGRs), Hinkley Point B in Somerset and Hunterston B in Ayrshire, last week made their first contributions to the national energy supply. Hinkley, now with an estimated capital cost of about £159 millions, generated only a little electricity before being stopped, pending further tests, but both are expected to build up to full power over the next few months.

**Mediterranean clean-up**

Experts from Mediterranean states,

meeting for two weeks in Barcelona under the auspices of the United Nations Environment Programme to discuss measures to clean up and prevent pollution of the Mediterranean, have received the text of a draft treaty embracing pollution from rivers and coastal establishments and exploitation of the sea bed, as well as dumping by ships and aircraft.

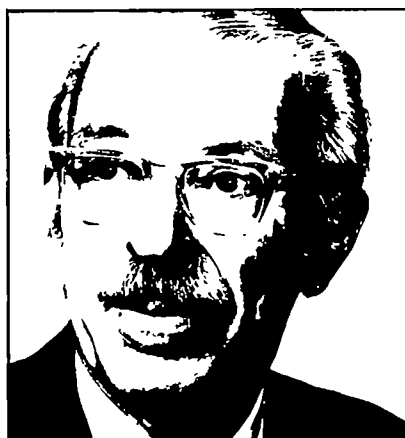
**Indian oil**

The Indian Government is to increase investment in oil exploration by a third to £200 millions as part of a bid to boost crude oil production. The target is self-sufficiency by the early 1980s, by which time the annual requirement is expected to be around 30 million tonnes. Though land-based wells at present produce less than a quarter of that figure, seismic surveys indicate potentially rich reserves in the offshore continental shelf. Optimistic interpretations even suggest that India could be an oil exporter within a decade.

THE toxicity, stability and pervasiveness of polychlorinated biphenyls (PCBs) as uncontrolled contaminants are known from a series of investigations that started in 1966, but very little has been done about it. Poisoning occurred in Japan in 1968 from eating rice oil containing PCBs. Clinical effects reported included stillbirths, undersized infants, bone and joint deformities and neurological disorders. Wholesale poisoning of chickens took place when PCBs leaked into fishmeal during processing. The contaminated feed produced low hatchability, and many newly-hatched chicks died. PCBs are distributed widely in major waterways in the USA; their presence led the Food and Drug Administration to warn against eating Lake Michigan fish, and the US Fish and Wildlife Service to call recently for eliminating all sources of PCBs in the environment within three years.

Apparently PCBs have escaped bans because they do not kill insects. They are frequently compared with DDT, which they resemble somewhat in chemical structure, and they can be mistaken for DDT in chromatography, but the resemblance ends there. PCBs are far more stable and more toxic to vertebrate organisms than DDT, which is broken down by enzyme systems that do not change PCBs. PCBs were in North Atlantic surface water at about 20 parts per  $10^{12}$  as compared with less than one part for DDT, even though the fall-out of DDT was estimated as twice that of PCBs. The Environmental

Protection Agency (EPA) is conducting a vendetta against DDT and other chlorinated hydrocarbon insecticides, but has soft-pedalled PCBs—perhaps because of pressure and influence on the EPA by the Environ-

**DDT: bystander or participant?**

THOMAS H. JUKES

mental Defense Fund (EDF), whose head scientist engagingly revealed EDF's motivation as follows: "If the environmentalists win on DDT, they will achieve, and probably retain in other environmental issues, a level of authority they have never had before. In a sense, then, much more is at stake than DDT".

The toxic effects of PCBs and mercury on wild birds may have provided a convenient weapon for clobbering

DDT. However, Scott and his collaborators at Cornell University simultaneously compared the effects of PCBs, DDT and methyl mercury on laying hens. Hatchability was reduced at 8 weeks from 92% in the controls to 50% with 10 p.p.m. of PCBs and to 2.4% with 20 p.p.m. DDT had no effect on egg production or hatchability at the highest level fed (100 p.p.m.), and increased the breaking strength of eggshells on a low-calcium diet. Methyl mercury, 10 p.p.m., adversely affected egg production, eggshell strength, fertility, and hatchability, and it increased morbidity and mortality. Adverse effects attributed to DDT, especially on eagles, have been reported in regions contaminated with mercury. The Cornell workers noted that many reports relating reproductive declines in wild birds to DDT and DDE were based on analyses that did not distinguish DDT from PCBs, and that some investigators had tried to reinterpret results in terms of PCBs.

DDT has never been recorded as having poisoned human beings through the food supply or by industrial exposure. Scott *et al.* say that "condemnation by correlation", in the case of DDT, "may have been downright dangerous to public health". In contrast to pesticides, whose transport and use are publicised, monitored and regulated, PCBs are unrestricted. They have been dumped out of discarded electrical capacitors and transformers, and put on roads to settle dust. The dust is now being stirred up again.



# correspondence

## Closure in Belgium?

SIR,—Due to a political decision, a research institute I head is about to be closed (*Institut de Recherche Interdisciplinaire en Biologie Humaine et Nucléaire*) and another to be severely handicapped (*Département de Biologie Moléculaire*, under Professor J. Brachet) at the University of Brussels. In a European country where the stability of employment is high and the turnover rate of personnel very low, this situation is highly unusual.

The Institute was created in 1963 to develop, in connection with clinical research units and with a sister group in Pisa (Italy), the use of radioisotopes in medical research and medicine. Since then, it has slowly evolved into what is now the *Institut de Recherche Interdisciplinaire* of the *Université Libre de Bruxelles*, a lab where physicists, chemists, biologists and MDs collaborate in biomedical research on many subjects (thyroid metabolism, cancer, cyclic nucleotides, lung physiology, theoretical biology, etc.).

Since its creation, the group has been subsidised by Euratom and the Universities of Brussels and Pisa. In 1969, Belgium did not participate any more in the Euratom programme of Nuclear Medicine. At that time, the government decided to support and create new research groups, "Actions Concertées", in the universities which would, like the Medical Research Council research units in Great Britain and the National Institutes of Health clinical research centres in the US, be catalysts for university research. This has worked well since then. The groups of Brussels were the first to be created and thus the first to arrive at the end of their five year contract.

This year, the government developed a new framework for research in which the whole structure of Belgian research is changed. This project has been criticised. One of its features is the concept of subsidising university research in proportion to the number of students. The government has agreed that the groups would fit in well in this new programme, but it has tied the granting of finance to the "Actions Concertées" to the approval of its programme. As the new project is now delayed, and as the contract is coming to its end, no financial support is planned for 1976. Thus the group is now, because of a political squabble, left "in the air".

Most of the permanent staff was not paid at the end of December. Thus in a few weeks the group might be disbanded. This situation has been much discussed in the Belgian press. The Minister insists that the Universities should take over temporarily, but the hard pressed university is financially and legally unable to do it. It is already badly burnt by a new "loi-programme" on finances, which includes a new subsidisation scheme for universities and which caused much turmoil and student demonstrations in the country in 1975.

Yours faithfully,  
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## Crisis in Italian universities

SIR,—I was impressed by the objectivity and completeness of Gillian Boucher's article (November 20, page 190). The causes of the Italian university crisis do not require further elucidation, still less comments like those of Giacomo Morpurgo (December 11, page 475). Morpurgo's letter, if published in an Italian magazine and not in an authoritative and widely circulated scientific journal such as *Nature*, would not deserve mention. His statements are ridiculous and untrue.

It is absurd to assert that the "intended aim" of the recent laws was to assure a permanent position for a large number of leftists "disposed to aggressive political activity". Morpurgo knows that these laws were promulgated by a Christian Democrat government which has ruled the country for thirty years.

After the results of the June 15 elections nobody should be surprised that the majority of university teachers are left wing. The intellectual class in Italy (with the exception of a few people like Morpurgo) was leftist long before June 15.

It is true that departments are invaded less frequently now than a few years ago. But this phenomenon is not confined to Italy. In every country student dissent is weaker than it was in 1968, for reasons much more serious than those invented by Morpurgo.

The recent laws have given tenure to a large number of university teachers and researchers whose positions had been precarious for a long time pre-

viously. The laws have repaired old injustices. If many teachers do not deserve their present positions, that is the fault of the University "baroni" who, by keeping their staff in menial positions for their own convenience, have given them the right to exploit the new laws. One of the privileges of the full professors was to create whatever positions they wanted and to assign them to whoever they liked for as long as they liked. If today the Department of Physics in the University of Genoa has more teachers than students, that, too, is the fault of Professor Morpurgo and his colleagues.

Yours faithfully,  
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ANNA GHIRETTI-MAGALDI  
*Institute of Animal Biology,  
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## Water to the Dead Sea

SIR,—While it would be possible to generate electric power (November 6, page 9) from the flow of seawater into any depression lying below sea level—the Dead Sea (surface at  $-390$  m), the Qattara Depression ( $-138$  m), or possibly even the Caspian Sea ( $-28$  m)—there is one thermodynamic possibility that applies to the Dead Sea alone. It is possible to deliver fresh water to the valley of the Dead Sea by a spontaneous process using a system with no moving parts.

The osmotic pressure of seawater is about 25 bar, and this pressure is reached with a head of 250 m of water. Any greater pressure would cause fresh water to flow through an osmotic membrane such as those used for desalination by reverse osmosis. Fresh water could be delivered to the valley of the Dead Sea not much below  $-250$  m. This water could then be used to irrigate a substantial area, particularly around the mouth of the Jordan River.

To use the flow of seawater into the Dead Sea exclusively for the generation of electric power is to neglect that what the desert most needs is fresh water. There is no reason, however, why the two projects cannot be combined, with fresh water being obtained at negligible added capital cost over that for electric power alone.

Yours faithfully,  
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# news and views

## Superfluid $^3\text{He}$ : magnetic pendula

from P. V. E. McClintock

OBSERVATIONS at La Jolla of nonlinear magnetic ringing seem to have demonstrated the existence of significant deviations from the predictions of "simple pendulum" models of dynamic magnetism in superfluid  $^3\text{He}$ . The experiments, by Webb, Sager and Wheatley (*Phys. Rev. Lett.*, **35**, 1010; 1975) can, however, be regarded as confirming the general correctness of the by now well-accepted pairing theory of the superfluidity.

In the picture of superfluid  $^3\text{He}$  which has emerged as a result of the last three years' intensive experimental and theoretical activity, liquid  $^3\text{He}$ -A, which is the stable phase for temperatures near 2.4 mK and pressures above 22 bar, can be considered as a mixture of three separate but interpenetrating components: two magnetically active superfluids capable of exhibiting non-dissipative flow, and a normal fluid component. One superfluid consists of pairs of atoms whose nuclei both tend to point parallel to a magnetic field (the up-spin superfluid) while the other is made up of pairs whose nuclei both tend to be antiparallel to the field (the down-spin superfluid). There are no pairs with opposed nuclear spins in the A-phase. The two superfluids behave almost independently of each other, being only weakly coupled by way of the nuclear dipole interaction. The coupling is, however, sufficient to allow pairs of atoms to pass from one superfluid to the other so that an equilibrium between their respective populations can be maintained.

Very interesting phenomena follow a small disturbance of this equilibrium by, for example, suddenly changing the magnitude of the applied magnetic field by a small increment: this slightly alters the chemical potential of the up-spin relative to the down-spin superfluid, and thus requires a transfer of pairs between them in order to establish a new equilibrium between their populations.

A striking consequence of Leggett's equations (*Am. Phys.*, **85**, 11; 1974) is that such a flow should be oscillatory in character. In fact, as both Leggett

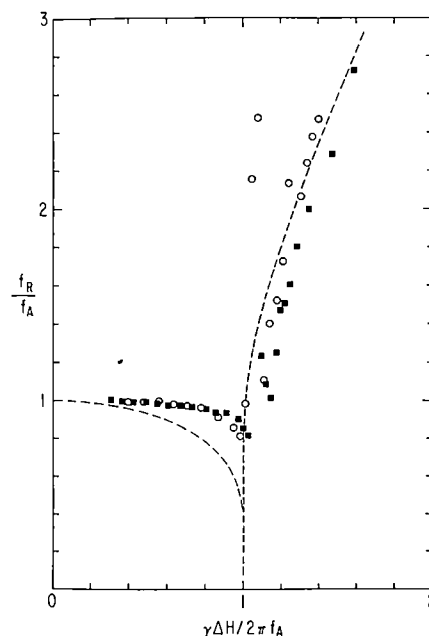
and also Maki and Tsuneto (*Prog. theor. Phys.*, **52**, 773; 1974) have pointed out, there is a remarkably close analogy with the a.c. Josephson effect, which involves an oscillatory transfer of pairs of electrons between two weakly coupled pieces of superconductor. In  $^3\text{He}$ , unlike the superconducting case, the two weakly coupled superfluids are not, of course, physically separated, being on the contrary completely interpenetrating. Nonetheless, the physics of the two situations, and the equations that govern them, are extremely similar: as in the case of the Josephson effect, it turns out that the equation which describes the flow of pairs between the up-spin and down-spin superfluids is formally identical with the equation describing the motion under gravity of a simple pendulum. As the result of small disturbances from equilibrium, one therefore expects that the system will oscillate with a characteristic frequency which is independent of amplitude: this internal Josephson effect or (as Richardson very reasonably suggested in his invited address to the LT14 conference) "Leggett effect" has been observed experimentally in a number of laboratories.

Larger disturbances from equilibrium should show non-linearities of precisely the same form as are exhibited by a simple pendulum. For example, one would expect a decrease in frequency at large amplitudes; and, for very large amplitudes, a frequency which is almost independent of potential energy, no matter whether this corresponds to the nuclear dipolar coupling in  $^3\text{He}$  or to the gravitational potential energy of the simple pendulum. The latter situation corresponds to the pendulum receiving such a substantial initial push that it goes "over the top" and rotates freely in the vertical plane. It is nonlinear effects of this nature which have been studied in some detail at La Jolla.

One of their experiments involved cooling some liquid  $^3\text{He}$  to just below the transition temperature  $T_0$  of 2.4 mK, under a pressure of 22 bar. The cell was a long rectangular cavity

with a cross section of 1 mm  $\times$  10 mm, and a static magnetic field was applied parallel to the longest walls: this approximated to an ideal geometry in which the highly anisotropic superfluid  $^3\text{He}$ -A should have taken up a well-defined texture. A small incremental change  $\Delta H$  in the magnetic field was made and a suitably positioned sensing coil was then used to detect the resultant oscillations (ringing) in the magnetisation of the liquid.

As expected on the basis of preliminary experiments and in the light of the theory, the frequency of the ringing was found to depend on the size of  $\Delta H$  which, in the pendulum analogy, represented the initial impulse given to the pendulum. Their result is shown in



Nonlinear ringing of the magnetisation of superfluid  $^3\text{He}$ -A, following a change  $\Delta H$  in the magnitude of the applied magnetic field (after Webb, Sager and Wheatley, *Phys. Rev. Lett.*, **35**, 1010; 1975). The initial ringing frequency  $f_R$  is divided by the characteristic A-phase frequency  $f_A$ , and plotted against  $\Delta H$  which has been normalised such that the anticipated zero in  $f_R$  should occur at unity on the abscissa. The dashed line represents a theoretical prediction, and is analogous to the behaviour of a simple pendulum.

Fig. 1. It can be seen that for small  $\Delta H$  the ringing frequency  $f_R$  remains close to the characteristic A-phase frequency  $f_A$ . For larger  $\Delta H$ ,  $f_R$  decreases, although not so fast as predicted by theory (the dashed line) and passes through a minimum. The latter situation is believed analogous to the pendulum having been given an initial impulse of exactly the right magnitude just to take it to the top of its orbit, where it therefore remains, which is why the theoretical curve indicates zero frequency. The facts that a minimum in  $f_R$  is observed rather than a zero, and that the minimum apparently occurs at slightly too large a value of  $\Delta H$ , may perhaps represent a genuine deficiency of the simple theory. The rapid rise in frequency just beyond the minimum seems, however, to be in reasonable agreement with expectation; and the so-called ringing-up and ringing-down phenomena which were observed close to the minimum constitute a striking verification of the general correctness of the pendulum model: experimentally, it was found that when the initial  $f_R$  was just to the left of the minimum in Fig. 1, the frequency increased (rang up) with time, corresponding to the increase in frequency of oscillation of a pendulum when frictional damping processes reduce the amplitude of its swing; and, for an initial  $f_R$  just to the right of the minimum, the frequency decreased (rang down) with time, corresponding to the frictional slowing down of a freely rotating pendulum.

Perhaps the physically most significant result of these experiments arises from measurements very close to  $T_c$  of the driven mode, corresponding to free rotation of the pendulum, which occurs

for very large  $\Delta H$ . Under these conditions  $f_R$  varies almost linearly with  $\Delta H$ ; with a constant of proportionality which is independent of the nuclear dipole forces, and is related only to the inertia of the rotating vector which is the analogue of the pendulum. The experimental results have turned out to be in excellent agreement with what would be expected on the basis of the already widely accepted pairing model of superfluidity in  $^3\text{He}$ .

The La Jolla group has also performed equivalent experiments for the other, low temperature, superfluid phase,  $^3\text{He-B}$ . Here, things are much more complicated because a third superfluid component, consisting of pairs of atoms with opposed nuclear spins, is also present. An equivalent pendulum analogy involves consideration of two coupled pendula. Maki and Hu have calculated (*J. Low Temp. Phys.*, **18**, 377; 1975) that there should be two zeros in  $f_R$ . The experiments have indeed demonstrated two rather shallow minima, but not occurring at the predicted values of  $\Delta H$ . However, the ringing up and ringing down phenomena could again be seen; and the frequencies of the driven mode were again in excellent agreement with the pairing model.

The quantitative discrepancies between the La Jolla data and predictions based on the conceptually very attractive pendulum models look real enough, but they will be treated with a certain amount of caution until the experiments have been repeated elsewhere: these measurements are being performed very close to the limits of present technology and the systems under study—magnetically active, anisotropic superfluids—are unique □

resource partitioning in communities of plants or animals have increased at four times this average rate, with a doubling time of less than 3 years. Our rough estimates indicate that work in theoretical ecology is generating papers at a rate similar to, if not greater than, this empirical work

In theoretical biology in general, and theoretical ecology in particular, this increase in papers is reflected in an increase in the number of journals—*Biometrika* (born in 1901), *Bulletin of Mathematical Biophysics* (1939), *Biometrics* (1945), *Journal of Theoretical Biology* (1945), *Mathematical Biosciences* (1967), *Theoretical Population Biology* (1970), *Journal of Mathematical Biology* (1974), *SIAM Journal on Applied Mathematics, Part C* (1976). This trend is faster than exponential, and indeed here a naive extrapolation will suggest an infinite number of journals within a few decades. This explosion in number of papers, and perhaps even in information, creates both human and financial problems.

Of these expanding numbers of theoretical papers in ecology, some contain insights of enduring use to theorist and empiricist, while others represent incremental advances in specialised areas. But essentially all those published are worthy of publication in some form. An established hierarchy in the importance of the various journals would be one rational response (and it exists to a certain extent), except that no publisher is keen to accept low place on the totem pole, and many reviewers seem to apply the same standards (sometimes too harsh, sometimes too lenient) to all work, regardless of the journal. This swelling flood of papers in theoretical ecology is exacerbated by traditions that allow mathematicians to present proofs and analysis in exquisite detail. Empiricists, on the other hand, are not allowed the analogous luxury of publishing their field notes. There is much to be said for innovative publishing practices whereby the major mathematical assumptions and conclusions are published, with the full proofs supplied in manuscript form to the referees and available as *samizdat* to those who care to write to the author. This practice could at least halve the bulk and cost of the journals listed above.

A variation of this idea, which has the advantage of conferring greater permanence on the material, is to publish the full paper in microform, and publish a lengthy abstract in conventional journal form. This is a logical outgrowth of the practice of putting journals into microform; for a review of contemporary trends, see Lynden (*Microform Review*, **4**, 15–24; 1975). In a recent survey (News and Com-

## The ecology of the ecological literature

from Judith May and Robert M. May

EVERYONE is familiar with the arrant nonsense produced by assuming that the current rate of population growth will continue indefinitely at its present value of about 2% per annum. If such a rate were sustained for around 5,000 years, the globe would be a ball of human protoplasm, expanding into space at the speed of light. This sort of naive extrapolation can be performed for various other quantities, many of which exhibit exponential growth rates faster than that of the human population as a whole. Thus if the rate at which the population of scientists grew in the 1960s were projected, in a mere two to three centuries there would be more scientists than people. Obviously such calculations are

not to be taken literally, but they do dramatise trends: in particular, the estimate above suggests that for the scientific community the growth rates of the 1960s were pathological, and that the slower growth rates of the 1970s are nearer the norm.

One of the basic products of scientists, namely published papers, has also grown exponentially in volume. Derek de Solla Price (in *Little Science, Big Science*, Columbia University Press, 1963) has documented a long term pattern of exponential growth in the number of scientific publications, with a doubling time of 10 to 14 years. Ecologists may rejoice or despair at Schoener's (*Science*, **185**, 27–39; 1974) observation that published studies of

ment in *Microform Review*, 4, 179–182; 1975) of 53 major publishing organisations representing 1,427 journals, roughly one third “expressed themselves in regard to how they would publish in microfiche.” Of these about one half were considering publishing the entire journal in microfiche but with a hard-copy version of contents and abstracts. Many of these publishers were also thinking of publishing the experimental details, appendices and other source and supplementary material only in microfiche and the abstracts or shorter versions of the articles in a paper edition. There are clearly many advantages to a two-tier system, with a conventional journal containing full summaries of the results, and with details published in microform (which could be produced directly from manuscript, saving time and expense). With contemporary techniques paper copies can be supplied on demand from a central microform holding.

Quite apart from the increasing number of journals, the rising production costs for individual journals create problems. One response for many journals has been to levy page charges, and sometimes to discriminate (often by slower publication) against those who cannot pay the charges. Van Valen (*Evolutionary Theory*, 1, 119–130; 1975) has recently made a tentative survey of various kinds of financial discrimination by journals catering to the broad area of ecology and evolution: of the various kinds of subtle and not-so-subtle discrimination he catalogues, the commonest is a mandatory charge for pages in excess of  $x$  (where  $x$  is typically around 10). Van Valen's figures suggest that of journals published in this general field in the USA, 69 of 112 manifest one or more discriminatory practices. The corresponding figures for Britain are 5 of 41; for the remainder of Europe 23 of 48, for Japan 6 of 8; for Canada 2 of

8. The average cost per 100 pages of the journal to the subscriber is roughly constant for the various countries (with commercial publishers based in Germany tending to be significantly higher), and for the various publishers.

Socially responsible readers of *Nature* will be relieved to know that Van Valen classes it as not discriminatory.

## Myelin structure

from N. P. Franks

New freeze-fracture studies by Pinto da Silva and Miller (*Proc. natn. Acad. Sci. U.S.A.*, 72, 4046, 1975) have questioned current concepts of myelin structure. Myelin is usually thought to be unlike any other plasma membrane. It has an unusually low protein content (about 20% by weight), very little enzymatic activity (Adams *et al.*, *J. Neurochem.*, 10, 383; 1963) and a lipid composition likely to result in a particularly stable bilayer (O'Brien, *Science*, 147, 1099; 1965). This is all consistent with the principal function of myelin being that of an electrical insulator of nerve axons.

The most widely accepted model of myelin structure is that of a basic lipid bilayer, with most of the protein distributed outside the hydrocarbon region. Support for this picture came from the freeze-fracture results of Branton (*Expl Cell Res.*, 45, 703, 1967), which showed that the fracture faces of frozen myelin were relatively smooth (similar to those seen with lamellar phases of lipids alone) in contrast to the particulate appearance of the fracture faces of other membranes. Since most membranes are thought to fracture within the hydrocarbon region, and since in some cases the particles have been shown to be protein-containing structures, Branton's results were consistent with a bilayer of lipids relatively uninterrupted by protein. More detailed information about the protein distribution has come from the X-ray diffraction work of Caspar and Kirshner (*Nature new Biol.*, 231, 46; 1971). Using an absolute electron density scale, previously established by Blaurock (*J. molec. Biol.*, 56, 35; 1971), they interpreted their electron density profiles as showing that the concentration of protein in the aqueous spaces between bilayers was 15–20% by volume (possibly higher near the lipid headgroups) with less than 10% by volume at the centre of the hydrocarbon region. The electron density profiles of Caspar and Kirshner impose important constraints upon models of myelin structure and were taken to support the notion that there was little

protein in the hydrocarbon region.

Surprisingly, Pinto da Silva and Miller now report a widespread distribution of particles on the freeze-fracture faces of myelin. They suggest that the previous observations of Branton were restricted to regions of high shadowing angle, where the particles were obscured. In untreated myelin, frozen shortly after dissection, the particle distribution was found to be uniform. If, however, the membranes were either fixed or glycerol impermeated then the particles aggregated and formed clusters in the plane of the membrane. Similar particle clustering has been observed in several other membranes, but what is remarkable about the photographs of Pinto da Silva and Miller is that the particle-rich regions in one membrane seem to line up with particle-rich regions in the adjacent membrane of the myelin sheath (this correspondence sometimes continuing across as many as 50 membranes). Furthermore, neighbouring membranes in the particle-rich regions seem more closely apposed than those in particle-free regions.

Although Pinto da Silva and Miller admit that the particle clustering itself is likely to be an artefact, they conclude that the correspondence between particle-rich regions from one membrane to the next does reveal an intermembrane interaction which exists *in vivo*. They argue, by analogy with other freeze-fracture studies on membranes, that the particles represent protein-containing structures which traverse the lipid bilayer. They suggest that these structures stabilise the myelin sheath by interactions across the cytoplasmic and extracellular spaces. Because the fracture faces are thought to represent an essentially hydrophobic environment, Pinto da Silva and Miller argue that the protein is likely to be apolar in nature and suggest the Folch-Lees proteolipid as a possible candidate. Indeed, the fact that about 80% of the myelin protein (Folch-Lees and Wolfgram proteolipids) can be solubilised in organic solvents argues for an apolar site for these proteins. It remains to be shown, however, that the particles observed by Pinto da Silva and Miller do represent protein-containing structures which are present *in vivo*. If this is the case then current models of myelin structure may have to be revised.

## Chemical antagonism in plant communities

from Peter D. Moore

ALMOST a hundred and fifty years ago De Candolle observed that the growth



### A hundred years ago

M. Berthelot, the celebrated chemist, is a candidate in the moderate Republican interest for the representation in the French Chamber of Deputies, of the district in which the Institute is situated.

from *Nature*, 13, February 10, 297; 1876.



of certain weeds was injurious to crops and postulated that the immediate cause of injury could have resulted from the excretion of toxins, possibly from their roots. Back in 1925, Massey (*Phytopathology*, **15**, 773; 1925) conducted experiments which involved planting such species as potato and tomato beneath the canopy of walnut trees and observed that wilting and death of the test plants occurred wherever they were in contact with the roots of the walnut, even when they were well clear of its canopy. He also showed that portions of bark from walnut roots could cause root death in water cultured tomatoes, thus indicating the involvement of some chemical factor rather than a competitive interplay for a mineral or water resource.

The importance of such chemical interactions between higher plants, sometimes termed allelopathy, is still probably underrated in ecological circles despite some valuable recent reviews, such as those of Whittaker and Feeney (*Science*, **171**, 757; 1971) and Rice (*Allelopathy*, Academic Press, New York; 1974). This has undoubtedly resulted from the general scarcity of information relating to chemical interactions in natural communities of plants. Many potential researchers must have been discouraged by the practical difficulties involved in designing experiments to test for allelopathic interactions, especially when one considers the physicochemical complexity of the soil in which such interactions occur and also the likely involvement of soil microbial communities. It may well be that some reported allelopathic agents are in fact the products of microbial degradation of the original plant tissues rather than the direct results of a plant's metabolism. Such a possibility, however, although of academic interest, does not preclude the evolutionary development of allelopathic competitive mechanisms which involve microbial symbionts. The fact that such mechanisms favour the host plants (and thereby favour the decomposer microbe) is of prime importance to the student of plant community dynamics. This being so, it should be possible to design simple experiments which do not attempt to disentangle the peripheral question of microbial involvement, but which are concerned with the overall outcome of plant-plant interactions involving allelopathy.

Just such an experiment has recently been reported by Newman and Rovira (*J. Ecol.*, **63**, 727; 1975) who have looked for chemical interactions between a number of common British grassland species. Four grass species and four herbaceous dicotyledons were chosen, none of which had given any field indication of allelopathic mechanisms at work. Plants were grown in

buckets of sand, together with an inoculum of soil from their original habitat; it is to be expected, therefore, that the normal rhizosphere microflora was present. Leachate of these pots (and of a control pot lacking plants) was applied to all other species examined and its influence upon the growth rates and mineral content of their shoots was examined.

The leachate from certain 'donor' species had a consistent and statistically significant effect in depressing the yield of receiver species. Yorkshire fog grass (*Holcus lanatus*), catsear (*Hypochaeris radicata*) and white clover (*Trifolium repens*) were most effective in this respect. Sweet vernal grass (*Anthoxanthum odoratum*) appeared to be the most sensitive species as a receiver of leachate, while *Trifolium repens* grew faster when supplied with almost any plant leachate than under the equivalent control conditions. *Anthoxanthum* was of additional interest in that its growth when supplied with its own leachate was faster than under any other conditions. Both *Holcus*, and the crested dog's tail grass (*Cynosurus cristatus*) also showed some growth stimulation when treated with their own leachate.

Although lack of rigorous replication in this experiment precludes detailed conclusions concerning the precise interaction of individual species, it is evident from these results that allelopathic mechanisms could be of considerable importance in determining composition and pattern in neutral grassland swards. Of particular importance is the demonstration that species differ in their toxic potential and also in their sensitivity as receivers of toxins. Self stimulation amongst grassland species could be of considerable importance in the attainment of dominance within a sward, whereas self sensitivity could lead to a wider spacing of individuals and an avoidance of intraspecific competition for resources within the population. Newman and Rovira's results, in this respect, agree well with the behaviour of their species in the field, but the observations of other workers are not so simply explained. For example, McNaughton has demonstrated auto-inhibition of seed germination in the reed mace, *Typha latifolia* (*Ecology*, **49**, 367; 1968), yet this is a species which can dominate extensive areas. The selective advantage of autotoxicity may operate in different ways in different species.

By far the most important point to emerge from Newman and Rovira's data, however, is that allelopathy is a potentially important mechanism for species interactions even in situations where such chemical devices have not previously been suspected. They effectively by-pass the ecologically

irrelevant question of microbial involvement; indeed, in using only young roots in their leachate production, they could be underestimating the overall influence of allelopathy in these species if there is a microbial intermediary involved. □

## Volcanic processes in ore genesis

from I. G. Gass

A packed lecture hall and an international range of contributors testified to the interest engendered by the recent joint meeting of the Institute of Mining and Metallurgy and the Volcanic Group of the Geological Society held in London on January 21-22 on the subject of Volcanic Processes in Ore Genesis. Seemingly, the timing and the topic were right and sufficient progress has been made in the area in recent years to attempt a synthesis.

Two major themes pervaded the meeting. On the one hand were the academics who, using trace element and isotope geochemistry and even naked thermodynamics, erected hypotheses and models for the tectonic setting, origin and circulation of ore-forming fluids. At the other extreme were those who provided the critical evidence as to whether these data applied to a particular ore deposit. Fortunately, speakers such as J. P. Hunt (Scripps Institution: ex-Anaconda), T. Sato (Japan Geological Survey) and G. Constantinou (Cyprus Geological Survey) with experience in both areas more than adequately bridged the all too common gulf between the two.

Stable isotope studies strongly suggest that the origin of the ore-carrying solutions was either seawater, in the case of massif sulphides formed at or near constructive margins, or meteoric waters for ore bodies such as porphyry coppers, emplaced above subduction zones. The role of magmatic waters is minimal in the former and minor in the latter; magmatic processes seem to provide the thermal energy and very little else. Both thermodynamic models of geothermal systems, as presented by J. W. Elder (University of Manchester), or modification of stable isotope ratios (E. T. C. Spooner, University of Oxford and T. H. E. Heaton, Kilbride) indicate that the circulation of the solutions that cause mineralisation follows immediately after the magmatic thermal event and that they are short-

lived in terms of thousands rather than millions of years and vigorous. There are therefore signs of a semi-quantitative breakthrough in the understanding of geothermal processes. This has been brought about by the use of a wide spectrum of analytical techniques on, for instance, the ophiolite massive sulphides of Cyprus and the porphyry copper of El Salvador. Their use elsewhere will markedly enhance our understanding of the processes involved in ore genesis.

Happily, the days when it was considered almost indecent for 'pure' academic scientists to concern themselves with 'ores' are over. A period of fruitful collaboration between hard-headed practicality and high quality academic research is, it is hoped, here to stay. □

## Phyto-insanitary Britain

from a Correspondent

A LARGE proportion of the money devoted to agricultural research in the UK is spent on phytopathology and crop protection and has enabled the production of virus-free and disease-resistant crops that can be fully protected by insecticides, fungicides and herbicides, to give near maximum possible yields.

Phytopathology research within the UK has been mostly confined to known indigenous pathogens but there is an additional threat from the vast amounts of plant material imported in many different forms every day. This comes from all over the world and frequently carries pathogenic organisms or strains of pathogens alien to this country. Fortunately most of these pathogens cannot survive in the unfavourable climate but the few that can may cause economically serious diseases.

Many such diseases have already entered the UK through the importation of infected plant material. Fire-blight of apples and pears, for example, was unheard of in Britain 20 years ago, though it was widespread in North America. It is now an established disease in southern England affecting hawthorns, many ornamental species of Rosaceae and posing a potential threat to apple and pear species, though disease outbreaks have been mostly confined to South-East England (*Ann. appl. Biol.*, **69**, 137; 1971). This disease has put severe restrictions on nurserymen who wish to export apple and pear varieties. Tobacco ringspot virus has also been introduced recently on pelargoniums imported from the USA.

The fruit industry suffered another setback recently when the Ministry of Agriculture reported that at least 150 acres of plums have some levels of infection with plum pox (sharka) virus, an aphid-borne virus. This potentially devastating disease originated in south-eastern Europe but has now spread throughout most of the continent. The Germans unknowingly introduced plum pox into their own plums from propagating material brought back from Yugoslavia after the last war, which resulted in serious losses in crop production in some regions. The virus is now endemic in many parts of Eastern Europe infecting a wide range of *Prunus* species (*Acta. Horticulturae*, **44**, 155, 1975).

Britain, through the Nuclear Stock Association (Tree Fruits), has been producing virus-free plums for nurserymen and fruit farmers for several years. Ironically a wide range of *Prunus* species from all over Europe is also imported even though the dangers of plum pox have been apparent for many years (*Pl. Pathol.*, **17**, 66, 1968). Much of this material is accompanied by phytosanitary certificates of the exporting countries which are not necessarily the countries of origin. These certificates state that the material has been inspected and is certified free of certain diseases.

Most virus diseases cannot be detected by visual inspection, however, particularly in dormant plants in transit. When the material arrives in the UK it is again inspected by the Ministry of Agriculture Plant Health Inspectorate who are not equipped to detect most virus infections at this stage. The plants are then distributed to nurseries for propagation, and are often grown close to virus-free rootstocks and mother-trees in the nuclear stock scheme. In the retesting of nuclear stocks carried out last year some plum pox infection was found.

As plum pox severely affects Victoria plum, the most widely grown cultivar in the UK, the survival of the plum industry is threatened unless steps are taken to eradicate this disease.

Because of the present loopholes in the system, which have been apparent for many years, particularly for virus diseases, it may only be a matter of time before diseases such as apple proliferation, oak wilt and pear moria are introduced.

Unlike countries such as New Zealand and the USA, the UK is failing to implement effective phytosanitary measures. Farmers and growers in Britain are not getting the protection they require and production is being restricted or made more costly by the introduction of new diseases. For too long exporting countries have been able to sell sub-

standard and infected plant material, undercutting local industries and threatening the health of home-produced material. It is time that the Ministry of Agriculture and Plant Health Branch heeded the warnings of research workers and implemented effective phytosanitary procedures. □

## Observing satellites

from a Correspondent

On January 17, 1976, there was an all-day meeting, at the Royal Society, in London, of optical observers of satellites and the university researchers who are, or soon will be, using their observations. The meeting was organised by the Optical Tracking Subcommittee of the British National Committee on Space Research, and the stimulus was the recent award by the Science Research Council to the Universities of Aston and Leicester of grants for research projects based on optical observations of artificial satellites. The current observing effort is to be augmented by recruiting a second team for the Hewitt Camera at Malvern and an increase in staff in the Satellite Orbits Group at the Appleton Laboratory, Slough, which provides the prediction service.

THE morning session began with an outline of the university proposals from their initiators, C. J. Brookes (University of Aston) and A. J. Meadows (University of Leicester). Brookes's programme includes a varied group of geophysical studies based on orbital analysis. The aims are to obtain better values of zonal harmonics in the geopotential, to study the variation of atmospheric rotation rate and air density during a solar cycle, and to study in detail the radiation pressure effects on orbits, using balloon satellites. Meadows proposed to concentrate on two topics: first, analysis of resonant lunisolar perturbations, by intensive observation of satellites at particular inclinations (56.06° being a promising starter); and second, studies of spin rates of similar satellites in orbits at different heights, to distinguish the effects of atmospheric and magnetic damping in the hope of measuring the variations in both.

After these plans for the future, some recent results from orbital analysis were presented. D. M. Brierly (Royal Radar Establishment, Malvern) described his determination of air density at a height of 128 km from a

THERE have been many studies of nucleon transfer reactions at low energies (5 to 50 MeV) and these have given much information on the single particle states of nuclei (see for example, *Nature*, **254**, 18; 1975; **256**, 615; 1975). Very few analyses have been made at higher energies, partly because it is then difficult to resolve the final states and partly because it is thought unlikely that such studies could give any information that could not be obtained from the technically much easier studies at lower energies.

In 1974 a group working at the Saturne synchrotron (*Phys. Lett.*, **52B**, 57) published the results of their measurements of the  $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$  neutron pickup reaction at 700 MeV. These data were remarkable because the energy resolution was sufficient to resolve several states of  $^{11}\text{C}$ , and thus provided the only stripping or pickup data available above 200 MeV.

The group made a preliminary analysis of their data using the distorted wave formalism, with the zero-range approximation for the neutron-proton force. At high energies, however, the momentum transfers are large, and this invalidates the approximation. They also ignored the D-state component of the deuteron wave function, although this is expected to contribute strongly at high energies. In spite of this, they found that the overall shape of the differential cross section is given quite well by their calculation, as shown in the figure, but the absolute magnitude is wrong by a factor of two.

In the past few years computer programs using a finite range neutron-proton force have been developed,

mainly to analyse heavy ion reactions. Rost and Shepard (*Phys. Lett.*, **59B**, 413; 1975) have now used one of these programs to analyse the  $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$  data, and have also included the effect of the D-state of the deuteron.

As shown in the figure, they found that the theory then gives a cross-section in good agreement with the experimental data, both in absolute magnitude and in overall shape. As expected the contribution of the D-state is dominant, so the previous agreement in shape must be largely fortuitous. It is thus very important

## Neutron pickup at high energies

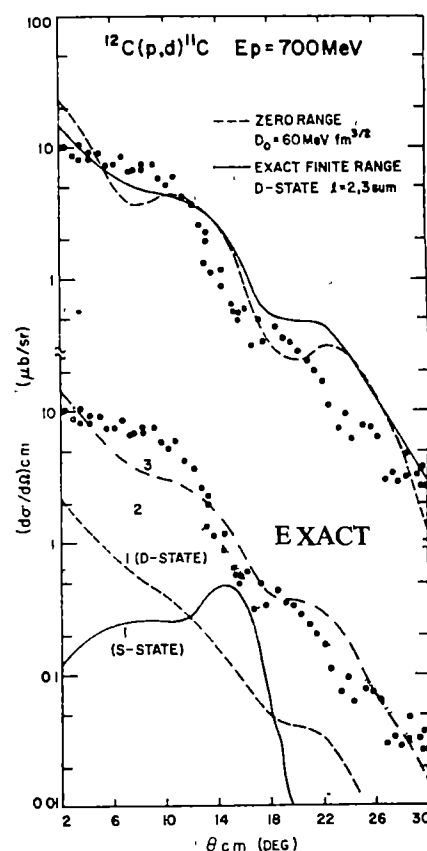
from P. E. Hodgson

for reactions of deuterons at high energies to use an accurate deuteron wavefunction, and the results can themselves provide information about that wavefunction.

This conclusion is reinforced by studies of the contribution of the S-state component of the deuteron wavefunction Rost and Shepard used an S-state waveform calculated from the Reid soft-core potential, that has been obtained by fitting a large body of nucleon-nucleon and deuteron data. Calculations with the Hulthén wavefunction, a simple analytical function widely used at low energies, gave cross sections completely in disagreement with the experimental data.

This work shows the value of repeating familiar analyses at sub-

stantially higher energies, whenever it is practicable to do so.



Differential cross section for the  $^{12}\text{C}(\text{p},\text{d})^{11}\text{C}$  reaction at 700 MeV compared in the top section with zero-range and exact finite-range distorted wave calculations. The zero-range curve has been arbitrarily divided by two to fit the data. The lower section shows the individual cross sections for the S-state  $L=1$  and the D-state  $L=1,2,3$  contributions.

decaying Molniya satellite, entirely on the basis of visual observations from Britain. Brookes presented the results of his first orbit determination, of Cosmos 373 (1970-87A) at 25 epochs between 1971 and 1975: this orbit, at inclination  $62.9^\circ$ , is closer to the critical inclination of  $63.4^\circ$  than any other orbit that has been accurately analysed, and he has determined the oscillation in perigee height caused by odd zonal harmonics in the geopotential as 59 km—the largest value yet established. D. G. King-Hele (Royal Aircraft Establishment, Farnborough) described new results obtained by himself and D. M. C. Walker in determining the atmospheric rotation rate from orbital analysis; the variations with height and local time have now been separated, and three curves of the variation of rotation rate with height were shown, for average local time, for evening (18-24 h) and morning (4-12 h).

The afternoon session began with an

excellent presentation by J. Eady (Ordnance Survey) of the work of the Hewitt Camera at Malvern, owned and operated by the Ordnance Survey. He commended the beautiful design of this accurate camera, a great tribute to the skill of the late Mr Hewitt, and looked forward to its increased use with a second observing team. The session continued with a succession of short contributions on topics connected with satellite observing. D. A. Richards (University College of Wales, Aberystwyth), offered some thoughtful comments on the slower time response of the eye in poor light, and demonstrated how the eyes see the linear swing of a pendulum as an ellipse if the light to one eye is reduced by a filter. The other contributions, to flash through them even more rapidly than they were presented, included a kaleidoscope of satellite shapes (J. A. Pilkington); an experimental demonstration of flash behaviour with satellite models (M. D.

Waterman); recent work on radio tracking of satellites at Kettering and associated stations (G. E. Perry); the telemetry of Soviet navigation satellites (C. D. Wood); a possible explanation of the break-up of the Pegasus balloon and several other US satellites (R. D. Eberst); the US Navy satellite tracking network (D. G. King-Hele); an analysis of a lengthy questionnaire on observing previously completed by the participants (M. D. Waterman); and a film of the Apollo-Soyuz Mission.

Apart from the formal presentations the meeting was intended to allow the visual observers to meet the new university researchers; to introduce the latter to some of the problems of observing; and to allow the volunteer visual observers to meet each other, to renew their enthusiasm, and to see how their unpaid work was utilised by the scientists. The subsequent comments of the participants suggested that these aims were fully achieved. □

# articles

## Head-tail radio sources in the cluster of galaxies Abell 1314

Jacques P. Vallée & Andrew S. Wilson\*

Sterrewacht, Leiden, The Netherlands

*A high resolution study of the cluster of galaxies Abell 1314 has revealed two 'head-tail' radio sources, associated with the galaxies IC708 and IC711. The tail of IC711 extends fully 820 kpc. The source properties are described and discussed in terms of the 'radio trail' model.*

THE 'head-tail' radio sources are characterised by a highly elongated radio structure, near one end of which lies the associated optical galaxy—usually a giant elliptical. The first examples of these tadpole shaped objects were discovered by Ryle and Windram<sup>1</sup> in the Perseus cluster, and roughly a dozen have now been recognised, mainly through observations with the aperture synthesis instruments at Cambridge and Westerbork. Most, perhaps all, of these sources are associated with clusters of galaxies. A compendium of currently recognised 'head-tail' galaxies is presented in Table 1, along with their more salient properties.

The most satisfactory explanation of these objects is the 'radio trail' hypothesis<sup>2</sup>. As the galaxy ploughs through a dense intracluster gas at supersonic speed, it is envisaged to eject pairs of plasmoids in opposite directions, as commonly assumed for 'normal' double radio galaxies. These blobs then brake by interaction with the intracluster medium, and form a trail behind the galaxy. Jaffe and Perola<sup>3</sup> developed two models based on this idea. In the first (the 'independent blob' model), the dynamic and radiative evolution of a series of ram pressure confined plasmoids was calculated. This model has difficulties with the spectral data and so they considered a second case (the 'magnetospheric' model), in which the galaxy is endowed with a strong magnetic field, drawn out into a magnetotail by the rapid motion through the surrounding gas. The double structure and symmetry of the tails<sup>2,4-7</sup>, and the detection of compact sources at the positions of the galaxies themselves<sup>5,7</sup>, speak strongly in favour of the 'radio trail' hypothesis; and the polarisation data on NGC1265 (ref. 9) indicate the magnetic field to be aligned more or less along the tail, as might be expected in models invoking galaxy motion through an intracluster gas (for example the 'magnetospheric' model).

It is important to discover more of these objects to attack the following problems: first do 'head-tail' galaxies occur preferentially in rich clusters? Much needed information on the elusive intergalactic medium could thus be obtained. Second, can the shape of the tails be completely accounted for in terms of the orbit of the galaxy in the cluster's gravitational field, or are additional processes (for example 'gales', or a buoyancy effect in the intracluster gas) required to explain the pronounced curvature observed in some sources? Third, is there any

tendency for these objects to occur in clusters which also contain other active radio galaxies (see ref. 4)? Fourth, are the energy losses of the relativistic electrons by synchrotron radiation, inverse Compton scattering, and adiabatic expansion so severe that particle acceleration is required a large distance from the parent galaxy?

We report here two new 'head-tail' galaxies (IC708 and IC711), both located in the cluster of galaxies Abell 1314, and discuss their structure and interrelationship. This work forms part of a continuing programme to search for such sources with the Westerbork Synthesis Radio Telescope (WSRT).

### Selection of objects and observations

Owen<sup>10,11</sup> has surveyed over 500 Abell clusters of galaxies with a resolution of 10' at  $\lambda = 21$  cm, and presented contour maps of sources with complex or multiple features. Although his map of the radio emission from Abell 1314 shows extended structure, the resolution is inadequate to delineate the nature of the source and determine unambiguously with which cluster galaxy, if any, it is associated. This field seemed a promising candidate for higher resolution investigation, so we observed it with the WSRT at  $\lambda = 49$  cm (610 MHz) and  $\lambda = 6$  cm (4,995 MHz). The former wavelength, where the resolution is  $\sim 1'$ , is ideal for the detection of faint, extended features, whereas the latter enables a higher resolution ( $\sim 6''$ ) investigation of the more intense, compact regions.

The WSRT and the procedure for data analysis have been fully described elsewhere<sup>12,13</sup>. Briefly, the instrument consists of twelve 25-m parabolic antennae along an east-west line. Ten of these (telescopes 0 to 9) are fixed and the other two (telescopes A and B) are moveable along a rail track. The signal from each of the ten fixed telescopes is correlated with that from each of the two moveable ones, resulting in twenty simultaneous baselines ranging from the separation 9-A to 9-B + (19 × 72 m) at intervals of 72 m. Fourier transformation and linear combination of the correlated signals yield maps of the four Stokes parameters<sup>14</sup>. The reception pattern of the individual paraboloids causes emission away from the field centre to be attenuated. For the extended radio structure associated with IC711 and IC708, the attenuation at  $\lambda = 49$  cm is always < 3%. At  $\lambda = 6$  cm, the maximum attenuation of the extended structure on the maps of IC711 and IC708 is 15% and 6% respectively. Both the contour maps and the quoted flux densities have been corrected for these small effects. The observational parameters, sensitivities and highest resolution obtainable are summarised in Table 2.

### Results at 49 cm and optical identifications

Figure 1a and b is a radio photograph and a contour plot of the brightness (total intensity) distribution in the central region of Abell 1314 at  $\lambda = 49$  cm (the cluster diameter is

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Table 1 Confirmed head-tail galaxies

Optical galaxy position (1950.0)	Name of head-tail galaxy	Name of cluster of galaxies	Cluster redshift ( $z$ )	Optical magnitude ( $m$ )	Flux density at $\lambda = 21$ cm (Jy)	Power at $\lambda = 21$ cm ( $10^{24}$ W Hz $^{-1}$ )	Angular size of galaxy tail (arc min)	Corresponding linear size (kpc)	First shown 'head-tail' by (reference)
02 h 55 m 47 s +13° 22'	4C13.17A	Abell 0401	(0.08)	—	0.38	10.0	6.5	780	20
03 13 25 +41 08	IC310	Abell 0426	0.0183	14	0.80	1.0	8.5	260	1
03 14 06 +41 16	C. & R. 15	Abell 0426	0.0183	15	0.03	0.04	2.5	80	2
03 14 56 +41 40	NGC1265	Abell 0426	0.0183	14	7.70	10.0	10.5	320	1
04 45 32 +44 55	3C129	Unnamed	0.0213	17	9.00	20.0	14.0	500	4
11 31 15 +49 20	IC708	Abell 1314	0.0335	14	(0.75)	(4.0)	1.5	80	this paper
11 32 03 +49 14	IC711	Abell 1314	0.0335	14	(0.55)	(3.0)	15.0	820	this paper
11 56 59 +28 11	NGC4869	Abell 1656	0.0230	14	0.45	1.0	4.5	170	21
12 15 49 +35 08	NGC6109	Zw1615+3505	0.0296	15	3.00	10.0	12.0	590	22
16 21 17 +38 02	NGC6137	Unnamed	0.0310	14	0.43	2.0	1.0	50	22
16 58 19 +32 39	4C32.52E	Abell 2241	(0.066)	16	0.20	4.0	1.5	150	23
17 12 05 +64 05	4CT64.20.1	Abell 2255	(0.07)	16	0.26	5.0	1.5	160	20
22 47 25 +11 21	NGC7385	Zw2247+1107	0.0268	13	2.30	7.0	12.0	530	8

Redshift values in parentheses are estimated from apparent magnitudes. Flux densities and powers at  $\lambda = 21$  cm in parentheses are estimated from scaling down the corresponding values at  $\lambda = 49$  cm using a spectral index of  $-0.7$ . A Hubble constant  $H_0 = 50$  km s $^{-1}$  Mpc $^{-1}$  and a deceleration parameter  $q_0 = 1$  have been used throughout to compute the powers at  $\lambda = 21$  cm and the corresponding linear sizes.

$\sim 2^\circ$ ). A highly elongated radio structure can be traced on a scale of  $15'$ , at the southern end of which, but still within the radio contours, lies the galaxy IC711. We identify the elongated feature as a 'head-tail' source associated with this galaxy, for the relative position of optical and radio features is typical of such objects and our observations at  $\lambda = 6$  cm (see next section and Fig. 2) show IC711 to be active. If the extended structure to the north of declination  $49^\circ 22'$  and elongated in position angle  $\sim 30^\circ$  is, in fact, a part of the tail and if IC711 is a member of the cluster, the overall length of the source is  $> 800$  kpc ( $H_0 = 50$  km s $^{-1}$  Mpc $^{-1}$ ). IC711 is then the longest 'head-tail' galaxy known, with a distance  $\sim 200$  Mpc. The tail of IC711 passes to the east of another well resolved, but more intense and compact, source at declination  $49^\circ 20'$ . This source is identified with the galaxy IC708, although the apparent peak of the emission at  $\lambda = 49$  cm is displaced some  $20''$  from the optical centroid. Observations of IC708 at  $\lambda = 6$  cm (Fig. 3) show, however, that it is both active and possesses a well developed radio trail, fully accounting for the displacement apparent in the lower resolution map at  $\lambda = 49$  cm. Webber<sup>15</sup> observed Abell 1314 at three radio frequencies and detected two sources which he identified with IC709 and IC711. Our observations show the former identification to be incorrect. Although the galaxy IC709 does lie close to the tail of IC711 (between IC711 and IC708), we do not detect it, allowing an upper limit for its flux ( $S_{49\text{ cm}} < 10$  mJy) to be established.

In the central part of the cluster, two other bright galaxies are detected at  $\lambda = 49$  cm. IC712, an elliptical galaxy with  $m_v \simeq 14$  (A. Oemler, personal communication), lies  $\sim 7'$

to the north of IC711, and coincides with an unresolved radio source (RA (1950.0) = 11 h 32 min 06.47 s, dec. (1950.0) =  $+49^\circ 21' 15.6''$ ) with flux density  $S_{49\text{ cm}} = 58$  mJy. The

Fig. 1 The brightness (total intensity) distribution in the central part of the cluster of galaxies Abell 1314 at  $\lambda = 49$  cm and resolution  $52'' \times 69''$ . *a*, Radio photograph (kindly made by W. Jaffe). *b*, Contour map. In *b*, the crosses show the positions of the optical galaxies, measured by Oemler (personal communication), and the half-power beam width is represented by the ellipse in the bottom right hand corner; the contour values are: 5, 10, 15, 25, 50, 100, 150, 200, 350 and 500 mJy (beam area) $^{-1}$ .

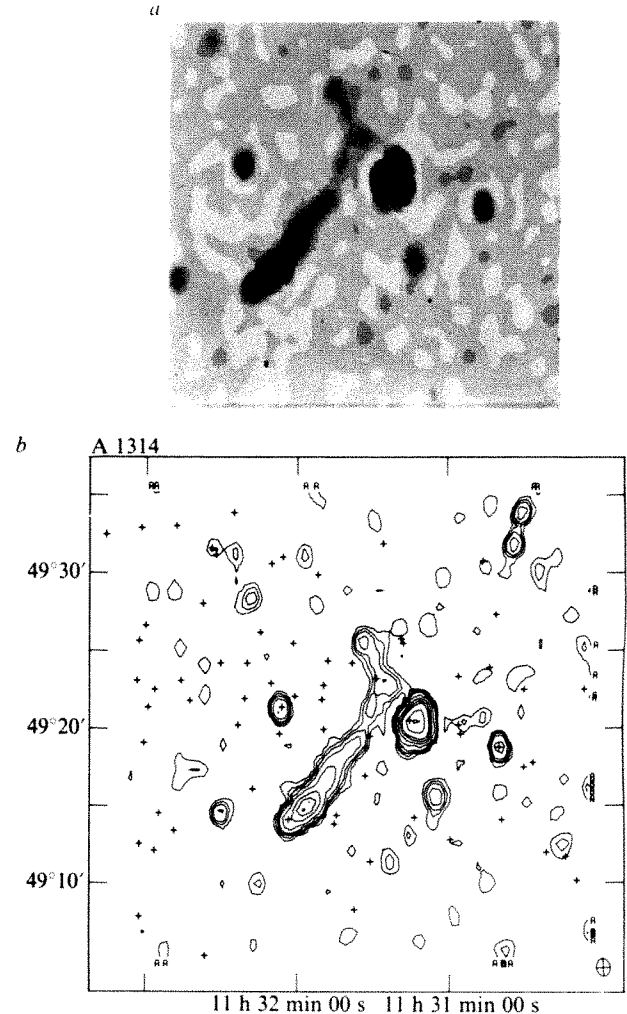
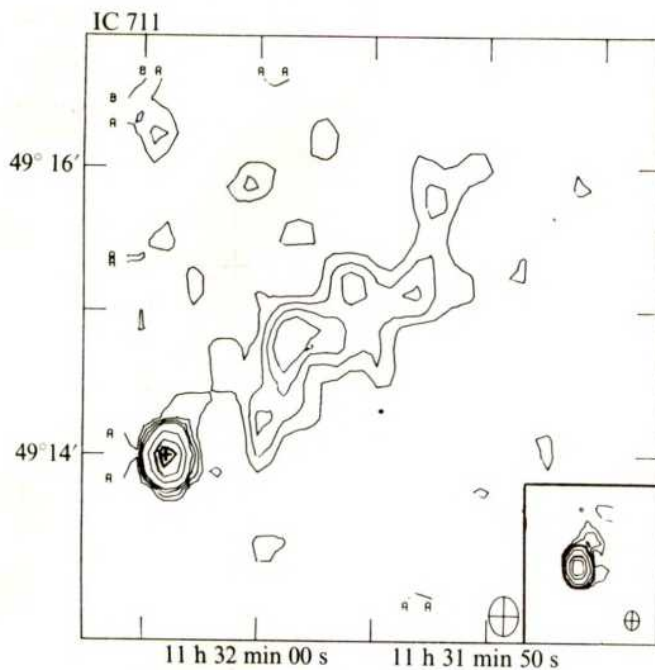


Table 2 Observational parameters

Galaxy observed	Whole cluster	IC708	IC711
Field centre RA (1950.0)	11 h 32 min 00 s	11 h 31 min 12 s	11 h 32 min 00 s
dec. (1950.0)	$49^\circ 20' 00''$	$49^\circ 20' 00''$	$49^\circ 14' 00''$
Wavelength (cm)	49	6	6
Observing time (h)	$1 \times 12$	$1 \times 12$	$1 \times 12$
Minimum baseline (m)	72	36	54
Spacing increment (m)	72	72	72
Observing period	August 1974	April 1975	March 1975
Highest resolution, RA $\times$ dec. (arc s $\times$ arc s)	$52 \times 69$	$6 \times 8$	$6 \times 8$
HPBW of individual paraboloids (arc min)	82	10	10
Radius of first grating response, RA $\times$ dec. (arc min $\times$ arc min)	$24 \times 32$	$3 \times 4$	$3 \times 4$
R.m.s. noise (mJy (beam area) $^{-1}$ )	1.6	1.2	1.2



**Fig. 2** The brightness (total intensity) distribution in the galaxy IC711 at  $\lambda = 6$  cm. The half-power beam width ( $12'' \times 16''$ ) is shown by the ellipse in the bottom right hand corner and the cross marks the centroid of the optical galaxy. The contour values are: 2.5, 5, 7.5, 10, 20, 30 and 40 mJy (beam area) $^{-1}$ . The inset in the bottom right hand corner shows the  $\lambda = 6$  cm nuclear source at a resolution of  $6'' \times 8''$ , with contour values: 1.25, 2.5, 3.75, 5, 10, 20 and 30 mJy (beam area) $^{-1}$ .

second galaxy ( $m_v = 15.7$ ) is a spiral with a flux  $S_{49\text{cm}} = 70$  mJy and radio position RA = 11 h 30 min 40.45 s, dec. =  $+49^\circ 18' 49.8''$ . If this last galaxy is a member of Abell 1314, its radio power at  $\lambda = 49$  cm is  $3 \times 10^{23}$  W Hz $^{-1}$ ; it is unresolved at  $\lambda = 49$  cm. Markarian 178, an object possessing a strong ultraviolet excess, lies  $\sim 14'$  north-west of IC712, and is not detected in our survey, implying that its flux  $S_{49\text{cm}} < 5$  mJy; its redshift ( $v_r = 270$  km s $^{-1}$ ) is much lower than that of the cluster, and Sargent<sup>16</sup> has suggested that it is a dwarf emission-line galaxy. A more complete discussion of the optical identifications of other radio sources in the field will be presented elsewhere.

## Results at 6 cm

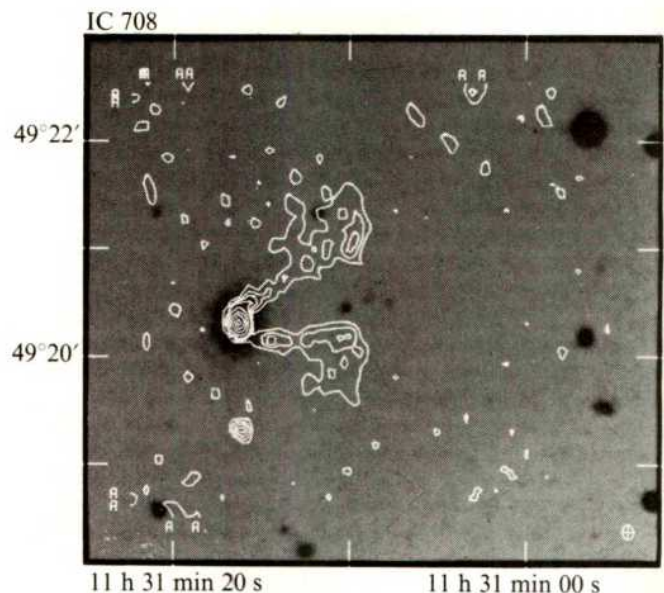
To explore the structure of the brighter parts of IC711 and IC708 with finer resolution, we have observed them with the WSRT at  $\lambda = 6$  cm. The map of IC711 with resolution  $12'' \times 16''$  (Fig. 2) shows an unresolved source, whose position agrees well with the centroid of the optical galaxy (as measured from the blue Palomar Sky Survey print) and is presumably to be identified with its nucleus. To the north-west of this source lies the tail, the brighter parts of which are detected up to  $\sim 3'$  from the galaxy. Between the brightest part of the tail and the nucleus, little radio emission is found. On the 'radio trail' hypothesis, this would imply that IC711 has not ejected double plasmoids recently. A close examination of the radio contours within  $10''$  of the compact source at a resolution of  $6'' \times 8''$  (see inset in Fig. 2) yields, however, evidence for a double feature apparently emerging from the nucleus. Although the brightness of this feature is only about twice r.m.s. noise, it is interesting that it is 'swept back' in the general direction of the tail itself. Basic data relevant to IC711 (and IC708) are summarised in Table 3.

The structure of IC708 at  $\lambda = 6$  cm is shown in Fig. 3. The intense resolved source on the  $\lambda = 49$  cm map (Fig. 1) superimposed on IC708 may now be seen to consist of four components. The brightest, unresolved at  $\lambda = 6$  cm, lies close

to the centroid of the optical galaxy (Table 3) and is, as in the case of IC711, presumably to be identified with the nucleus. Stretching  $> 1'$  to the west of the nucleus are two well resolved strands, implying that IC708 is also a 'head-tail' galaxy. There is no reason to believe that the fourth component,  $\sim 1'$  of the nucleus, is physically related to IC708. The nuclear source is weakly (three times r.m.s. noise) linearly polarised (see Table 3).

## Discussion

Our observations of IC708 and IC711 are consistent with the 'radio trail' model in its general form<sup>2</sup>. The observations at  $\lambda = 6$  cm show the two galaxies to be active, implying that the nucleus is the supplier of energy in both cases, as is known to occur in 'normal' radio galaxies. The source morphologies strongly suggest a radio trail, particularly for IC708. A more careful scrutiny of the properties of IC711, however, reveals a difficulty with the simple models developed by Jaffe and Perola<sup>3</sup>. This problem centres around the large size of the source, an aspect we now discuss.



**Fig. 3** A contour map of the brightness (total intensity) distribution in IC708 at  $\lambda = 6$  cm and resolution  $6'' \times 8''$ , superimposed on the blue Palomar Sky Survey print. The half-power beam width is shown by the ellipse in the bottom right hand corner, and contour values are: 2.5, 5, 7.5, 10, 25, 50, 75 and 100 mJy (beam area) $^{-1}$ .

In both the 'independent blob' model and the 'magnetospheric' model, the relativistic electrons are envisaged to be accelerated within the optical galaxy rather than in the tail itself. We now show that severe difficulties result from this assumption in the case of IC711. An electron radiating at  $\lambda = 49$  cm in a typical equipartition magnetic field in the tail of  $4 \times 10^{-6}$  gauss has a half life to synchrotron radiation and to inverse Compton losses on the microwave background of  $t_{1/2} = 9 \times 10^7$  yr. Writing  $L$  = length of tail = 820 kpc, the average velocity of propagation  $V$  of such electrons with respect to the galaxy must be  $\geq L/t_{1/2} = 8,600$  km s $^{-1}$ , if they are accelerated within the galaxy. In the 'independent blob' model, the maximum possible  $V$  is simply the velocity of the galaxy with respect to the intracluster gas  $V_g$  (since ram pressure eventually brings the plasmoids to rest), which, if the galaxy is a member of the cluster, is surely much smaller than the above value. The value of  $V$  appropriate for the 'magnetospheric' model is less obvious since, although it is to be expected that the relativistic particles propagate through the thermal gas at about the Alfvén speed  $V_a$  (refs 17 and 18), the velocity of the thermal plasma in the



tail  $V_t$  with respect to the galaxy is unknown. Jaffe and Perola took  $V_t = V_g$ , so the maximum possible is  $V = V_g + V_a$ . The assumption  $V_g \ll 8,600 \text{ km s}^{-1}$  then implies  $V_a \geq 8,600 \text{ km s}^{-1}$ , that is, a thermal particle density  $\rho \leq 2 \times 10^{-30} \text{ g cm}^{-3}$ . Implicit in the 'magnetospheric' model is a rather uniform magnetic field aligned along the tail, and, with such a low gas density, appreciable polarisation could be expected at  $\lambda = 49$

duration of past activity in IC711 itself, presuming the intra-cluster gas to have a distribution similar to that of the galaxies. The much shorter length of the tail of IC708 could be accounted for if it has just entered the western edge of the dense gas. The apparent expansion of the two strands of IC708 near RA = 11 h 31 min 11 s (see Fig. 3) is indeed suggestive of a reduced gas density there.

Table 3 Some data on IC711 and IC708

Source parameter	IC711	IC708
Positions (1950.0): radio nucleus	RA = 11 h 32 m 03.90 $\pm$ 0.05 s dec. = 49° 13' 57.8 $\pm$ 0.6''	RA = 11 h 31 m 16.27 $\pm$ 0.05 s dec. = 49° 20' 19.3 $\pm$ 0.6''
centroid of optical galaxy*	RA = 11 h 32 m 03.95 $\pm$ 0.1 s dec. = 49° 13' 56.5 $\pm$ 1.0''	RA = 11 h 31 m 16.36 $\pm$ 0.1 s dec. = 49° 20' 18.1 $\pm$ 1.0''
Visual magnitude	14.8	14.4
Angular size of galaxy-tail at $\lambda = 49 \text{ cm}$ (arc min)	15	1.5
Corresponding linear size in plane of sky at $\lambda = 49 \text{ cm}$ (kpc)†	820	80
Linear size of nucleus (FWHM) at $\lambda = 6 \text{ cm}$ (kpc)‡	< 3	< 3
Flux densities (mJy): total, $\lambda = 49 \text{ cm}$	1,047 $\pm$ 100	1,432 $\pm$ 70§
nucleus, $\lambda = 6 \text{ cm}$	44 $\pm$ 3	114 $\pm$ 5
Linear polarisation of nucleus: percentage (%) at $\lambda = 6 \text{ cm}$	< 6	3.6 $\pm$ 1.2 (r.m.s.)
position angle (°) at $\lambda = 6 \text{ cm}$	—	134 $\pm$ 10
Power at $\lambda = 49 \text{ cm}$ (W Hz <sup>-1</sup> )†	$5 \times 10^{24}$	$7 \times 10^{24}$
Radio luminosity (W)†	$5 \times 10^{34}$	$8 \times 10^{34}$
Minimum energy in tail (erg)‡	$5 \times 10^{58}$	$1 \times 10^{58}$
Equipartition magnetic field in tail (gauss)‡	(2–6) $\times 10^{-6}$	$7 \times 10^{-6}$

\*Measured from the blue Palomar Sky Survey print with the 'Coradigraph' measuring machine at the Royal Greenwich Observatory.

†Assuming  $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ , deceleration parameter  $q_0 = 1$ , and a radio window extending from 10 MHz to 100,000 MHz.

‡Minimum energy estimates have been made assuming the total energy in cosmic ray protons to be equal to that of the electrons.

§Preliminary results at  $\lambda = 21 \text{ cm}$ , when combined with those at  $\lambda = 6 \text{ cm}$ , indicate that the background source 1' to the south of the nucleus of IC708 (see Fig. 3) has a spectral index  $\alpha = -0.6 \pm 0.1$ . Extrapolation of this spectrum to  $\lambda = 49 \text{ cm}$  yields  $S_{49\text{cm}} = 55 \text{ mJy}$ . The flux quoted for IC708 represents the flux of the whole source on the  $\lambda = 49 \text{ cm}$  map minus the above flux of the background source.

cm, since the Faraday rotation would be very low. Our data, however, show the tail of IC711 to be < 3% linearly polarised at  $\lambda = 49 \text{ cm}$  in the brighter parts. Of course, a more random magnetic field could account for the lack of polarisation, but in that case relativistic electrons should diffuse down the tail more slowly. These arguments suggest that the relativistic electrons radiating at  $\lambda = 49 \text{ cm}$  can probably not propagate from the nucleus to the end of the tail in the face of the energy losses. This conclusion is true *a fortiori* if the tail does not lie in the plane of the sky, if the magnetic field is stronger or weaker than the assumed equipartition value, or if adiabatic losses are also included. Our discussion implies, therefore, that the relativistic particles may be reaccelerated within the tail of IC711 itself, or somehow replenished from the intracluster medium. The latter possibility has been proposed by W. A. Christiansen (personal communication).

The size of IC711 and IC708 visible on the Palomar Sky Survey red print are at least 25'' and 65'' respectively. Examination of Figs 2 and 3 shows that the radio-emitting plasmoids are already swept back when < 10'' from the nuclei, well within the optical extents. A similar conclusion may be drawn from the  $\lambda = 6 \text{ cm}$  map of NGC1265 (ref. 7), and constitutes rather direct evidence that the intracluster gas blows through the galaxies, probably sweeping out any pre-existing gas, as has been proposed on theoretical grounds<sup>19</sup>. This conclusion depends only on the validity of the 'radio trail' hypothesis in its most general form.

The size (FWHM) of the distribution of optical galaxies in Abell 1314 is, after correction for the background galaxies,  $\sim 20'$  and  $15'$  in RA and dec. respectively. The latter value is close to the length of the tail of IC711, and we speculate that the extent of its radio trail is determined by the scale of the intra-cluster gas necessary to contain the plasmoids, rather than the

It is intriguing to note the change in position angle of elongation in the tail of IC711 at dec. = 49° 22', quite near IC708. There is, however, no definite evidence for any interaction between the two sources, and the peculiar morphology of IC711 could be accounted for in terms of projection effects, an elliptical orbit through the cluster being viewed obliquely.

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# T4 gene 32 protein model for control of activity at replication fork

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*Limited hydrolysis of gene 32 protein by various proteinases results in the production of three stable cleavage products. Two of these products show an affinity for native T4 DNA cellulose that the uncleaved protein does not exhibit. A model for proteolytic cleavage and for the local unwinding of DNA in advance of the replication fork is discussed in terms of this unusual binding affinity.*

GENE 32 protein (P32) of bacteriophage T4, isolated and characterised by Alberts *et al.*<sup>1</sup>, is essential for DNA replication<sup>2</sup> and recombination<sup>3,4</sup>. It has the unique ability to convert duplex DNA to single strands at a temperature that is 40 °C below the melting temperature ( $T_m$ ) of the DNA and is therefore thought to be responsible for local unwinding of duplex DNA in advance of the replicating fork. This ability to denature duplex DNA results from strong cooperative binding of P32 to single strands with little or no binding to double-stranded regions. Preferential binding of P32 to single strands, besides melting the duplex, has the reverse effect of facilitating base-pair alignment between single strands. Presented with single-stranded DNA, P32 induces a chain conformation (in the DNA) which increases the reannealing rate of homologous strands, a reaction which might be important to recombination.

Direction of the reaction denaturation $\longleftrightarrow$ renaturation in the presence of P32 depends on stability of the duplex. At levels of ionic strength and  $Mg^{2+}$  concentration presumed to be present *in vivo*, the  $T_m$  of T4 DNA would be about 85 °C and at physiological temperatures the presence of P32 alone would be inadequate to keep the duplex apart in advance of the moving replication fork.

A protein of molecular weight 27,000, which copurified with P32 (35,000) has been shown to denature T4 DNA and bind cooperatively to the denatured DNA. In the same conditions P32 could do neither<sup>5</sup>. This protein was named P32\* because it was derived from P32. Here, we show that production of P32\* is the result of a protease contaminant introduced during purification and that treatment of P32 with chymotrypsin, trypsin or pepsin, each produces P32\*-like molecules (27,000) and two other partial hydrolysates (34,000 and 26,000). We tested the binding of these limited hydrolysis products to single- and double-stranded DNA and found different combinations of affinities for each. A possible relationship between proteolysis and the binding/denaturing abilities of these products is discussed in terms of a model of reversible denaturation of duplex DNA.

## Origin of proteolytic activity in purified P32 preparations

On storage of purified P32 at 4 °C, a second component of lighter molecular weight (27,000) increased at the expense of P32 (ref. 5). This suggested that a protease

contaminant had copurified with P32 and was slowly producing P32\* from P32 by limited hydrolysis.

Three possible sources of proteolytic contamination were examined: a protease from *Escherichia coli*, a T4-induced protease, or contamination of pancreatic DNase I used during the preparation of cell extracts from which P32 was purified. T4 is known to induce a proteolytic activity related to processing of its head precursor proteins. This activity is present in lysates from some head negative (gene 23) mutant-infected cells and is absent in gene 21 mutant-infected cells<sup>6</sup>.

Purified <sup>35</sup>S-labelled P32 was treated with cell extracts from: *E. coli* cells infected with a gene 23 mutant (*amB17*); *E. coli* cells infected with a gene 21 mutant (*amN90*); uninfected *E. coli* cells; or a solution of pancreatic DNase I. Results are shown in Fig. 1; only the DNase solution produced any appreciable amount of proteolysis as judged by a decrease in molecular weight after 1 h incubation at 37 °C.

Commercially available DNase is known to be contaminated with chymotrypsinogen B (ref. 7). Purification of the DNase to remove chymotrypsinogen B (ref. 7) before preparation of cell extracts resulted in isolation of P32 with a greatly reduced amount of copurifying P32\*. Incubation of purified <sup>35</sup>S-labelled P32 with DNase pretreated with tosyl-phenylalanyl-chloromethyl-ketone (TPCK) caused substantial reduction in the amount of hydrolysis products formed. Therefore it seems likely that hydrolysis of a trace amount of chymotrypsinogen B to chymotrypsin B was mainly responsible for the P32\* found in aged P32 preparations.

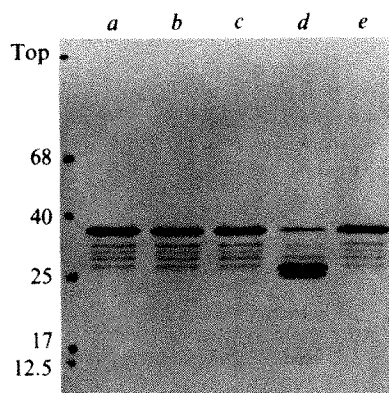
## Limited hydrolysis of P32 with known proteinases

Having established that production of P32\* resulted from protease contamination, we decided to try and produce P32\* from P32 by proteolysis with known proteinases. If this could be done then we could design and undertake experiments to determine why P32\* is able to denature T4 DNA and P32 cannot.

Treatment of P32 with three proteinases, bovine- $\alpha$ -chymotrypsin, bovine trypsin, and bovine pepsin in varying conditions (substrate concentration 0.2 or 0.86 mg ml<sup>-1</sup>; enzyme concentration 0.1–100  $\mu$ g ml<sup>-1</sup>; temperature 4 or 37 °C; reaction time 1 h at pH 8.1) produced three products: P32\*-I, -II and -III with molecular weights of 27,000, 34,000 and 26,000 respectively (for mechanism, see discussion under model for proteolysis). Conditions needed to reach a specific stage of hydrolysis differed with enzyme used but there was a definite order of appearance and disappearance of the three products. This order together with the position of each product after electrophoresis in SDS gels (12.5% acrylamide) is presented in Fig. 2.

In general, in the mildest conditions, P32\*-I, a molecule with the same mobility as that of P32\* appeared. With increasing enzyme concentrations two new products,





**Fig. 1** Autoradiogram of  $^{35}\text{S}$ -labelled gene 32 protein analysed on SDS gels. P32 treated with: *a*, T4 *amb17* (gene 23)-infected *E. coli* cell extract; *b*, *ambN90* (gene 21)-infected cell extract; *c*, uninfected cell extract; *d*,  $100\ \mu\text{g ml}^{-1}$  pancreatic DNase I (Worthington); *e*, untreated control. Markings with corresponding numbers along left-hand side denote position and molecular weight ( $\times 10^3$ ) of reference proteins marked with radioactive ink before autoradiograms were taken. 68, bovine serum albumin; 40, ovalbumin; 25, chymotrypsinogen; 17, myoglobin; and 12.5, cytochrome *c*. Treatment of  $^{35}\text{S}$ -labelled P32 with cell extracts:  $^{35}\text{S}$ -P32 was added to the cell extracts to give a final concentration of  $200\ \mu\text{g } 2.5 \times 10^5\ \text{c.p.m. ml}^{-1}$ . After incubation of protein with cell extracts for 1 h at  $37^\circ\text{C}$ , an equal volume of SDS sample buffer ( $0.125\ \text{M}$  Tris-HCl, pH 6.8, 10% 2-mercaptoethanol, 4% SDS $^{12}$ ) added and mixture frozen until SDS gel electrophoresis. SDS gel electrophoresis: Proteins were analysed on 12.5% polyacrylamide slab gels $^{13}$  containing 0.1% SDS using a discontinuous buffer system $^{12}$ . Proteins, in sample buffer, were denatured by heating samples in boiling water for 2 min. Samples were loaded on to gels and electrophoresis carried out at a constant current of 15 mA for 4 h. Gels were fixed in 30% TCA, stained with 0.1% Coomassie blue in 30% TCA, and destained in 7.5% acetic acid. Gels were dried and autoradiograms taken.  $^{35}\text{S}$ -labelled P32 preparations: *E. coli* B $^{\text{F}}$  grown to  $4 \times 10^8\ \text{cells ml}^{-1}$  in 200 ml low sulphate M9 medium (sulphate concentration  $5 \times 10^{-5}\ \text{M}$ ) was infected; after 8 min superinfected for a total multiplicity of ten, with T4 *amb17*292 (gene 55) a mutant which overproduces P32 (ref. 15). At 10 min  $^{35}\text{S}$ -sulphate (final concentration  $30\ \mu\text{Ci ml}^{-1}$ ) was added. After 90 min cells were collected and frozen. Purification of  $^{35}\text{S}$ -P32 was same as for non-radioactive P32, except DEAE-cellulose chromatography omitted. Non-radioactive P32 added to final concentration  $200\ \mu\text{g ml}^{-1}$  to act as carrier. Non-radioactive P32 preparations: *E. coli* B $^{\text{F}}$  grown to  $4 \times 10^8\ \text{cells ml}^{-1}$  in M9A medium was infected as described above. After 90 min cells collected and frozen until needed. Purification of P32 as described previously $^1$ ; 50 g cells yielded about 35 mg purified P32. Undigested P32 (both radioactive and non-radioactive) used in this experiment and those in Fig. 3 contained a small amount of P32\* and three additional bands between the position of P32\*-II and P32\*-I (see text). These three minor components were absent when P32 was prepared using DNase pretreated with diisopropylfluorophosphate (DFP) followed by hydroxylapatite column chromatography to remove contaminating chymotrypsin B (ref. 7); therefore, they probably are intermediates of P32 digestion by chymotrypsin B to produce P32\*. Cell extracts: Cultures (100 ml) *E. coli* B $^{\text{F}}$  in M9A medium grown at  $37^\circ\text{C}$  to  $5 \times 10^8\ \text{cells ml}^{-1}$  infected with T4 *amb17* (gene 23) or *ambN90* (gene 21) and superinfected 5 min later for a total multiplicity of eight. Cells collected by low speed centrifugation 30 min after infection. Cell pellets suspended in 5 ml sonication buffer $^1$  and sonicated. After high speed centrifugation to remove cell debris, supernatants used to treat  $^{35}\text{S}$ -P32. Uninfected cell extract prepared in same way.

P32\*-II and -III appeared. (The position of P32\*-II after electrophoresis in SDS gels was too close to that of P32 to detect a small amount of P32\*-II in the presence of a large excess of P32. Therefore, the fact that P32\*-I was seen first does not mean that P32\*-II was produced after P32\*-I, but rather P32\*-II was not seen until the amount of P32 was reduced.) With further increases in enzyme concentration and/or reaction temperature, P32 then P32\*-II and then P32\*-I disappeared. Several examples showing each stage of hydrolysis are presented in Fig. 3.

Pepsin was least efficient of the three proteinases probably because it was used at a suboptimal pH;  $1\ \mu\text{g ml}^{-1}$  was

required to produce a small amount of P32\*-I (column 2, Fig. 3). With higher concentrations of pepsin, stage *c* (Fig. 2) hydrolysis was reached but hydrolysis did not proceed beyond this point. Chymotrypsin with an activity midway between that of pepsin and trypsin produced any stage of hydrolysis indicated in Fig. 2 with  $1\ \mu\text{g ml}^{-1}$  producing stage *c* as shown in column 4 of Fig. 3. Trypsin, with the strongest proteolytic activity of the three proteinases, produced stage *c* and stage *d* hydrolysis at concentrations of  $0.1$  and  $1\ \mu\text{g ml}^{-1}$ , respectively. Chymotrypsin and trypsin at concentrations higher than  $10\ \mu\text{g ml}^{-1}$  and at  $37^\circ\text{C}$  were able to digest P32\*-III to even shorter peptide fragments.

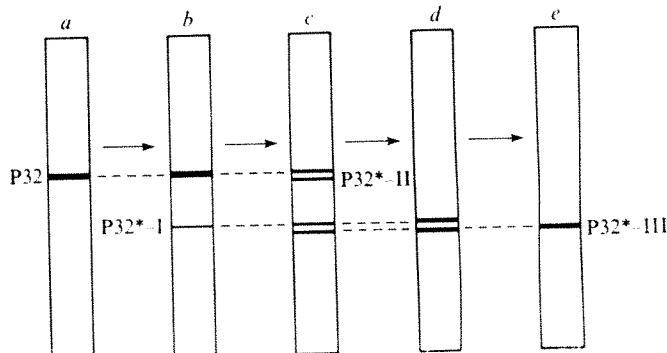
### Affinity of proteolysis products of P32 for single-stranded DNA-cellulose

Affinity of the limited hydrolysis products for single-stranded DNA was examined by chromatography on single-stranded (calf thymus) DNA-cellulose columns. Limited digests containing all four components (P32, P32\*-I, -II and -III) in  $50\ \text{mM}$  NaCl were loaded on columns of single-stranded DNA-cellulose and eluted off with increasing concentrations of NaCl (Table 1). Regardless of which proteinase was used for digestion, products with the same molecular weights behaved the same way. All four species adsorbed at  $50\ \text{mM}$  NaCl. Both P32 and P32\*-I showed strong binding to the single-stranded DNA with elution of either requiring a concentration of NaCl higher than  $0.6\ \text{M}$ . On the other hand both P32\*-II and P32\*-III showed a drastic loss of affinity as both eluted off the column with  $0.4\ \text{M}$  NaCl.

### Affinity of chymotrypsin-digested products for native T4 DNA-cellulose

It has been shown that P32\* can denature T4 DNA $^5$ . P32\* bound strongly to duplex T4 DNA-cellulose, whereas P32 did not (J. H. and C. Brack, unpublished). Presumably P32\* is also able to denature duplex T4 DNA bound to cellulose and then bind to single-stranded regions. Binding of P32 and the limited hydrolysis products of chymotrypsin digestion to native T4 DNA-cellulose was examined to see if the products showed affinity to T4 DNA-cellulose as P32\* did. Chymotrypsin was used because its activity is midway between that of trypsin and pepsin (Fig. 3). Digests containing all four components were loaded on to columns of T4 DNA-cellulose in  $5$  or  $25\ \text{mM}$  NaCl at  $4$  or  $20^\circ\text{C}$ . Results are summarised in Table 1b. In these conditions neither P32 nor P32\*-II showed any binding to T4 DNA-cellulose. P32\*-I and P32\*-III both bound in the four sets

**Fig. 2** Diagrammatic representation of position of limited hydrolysates produced by chymotrypsin, trypsin or pepsin, after electrophoresis on SDS gels. *a*, Untreated P32; *b-e*, limited proteolysis products with increasing enzyme substrate ratio or with increase of reaction temperature.



of conditions listed above. P32\*-III eluted at NaCl concentrations of 0.1–0.2 M. P32\*-I had stronger binding, as a NaCl concentration of 0.4 M was necessary for elution. This strong binding of P32\*-I is similar to the binding reported earlier for P32\* (ref. 5). The implication is that P32\*-I is capable of denaturing T4 DNA and binding cooperatively to it. The weaker binding of P32\*-III suggests that it is binding in a different manner from P32\* or P32\*-I.

### Model for limited proteolysis (Fig. 4)

Regardless of proteinases used the products were indistinguishable by SDS gel electrophoresis and chromatography of DNA-cellulose, yet these proteinases each have a different specificity for the bond they hydrolyse: trypsin is specific for the carboxyl group of lysyl and arginyl residues; chymotrypsin for the carboxyl group of tryptophanyl, phenylalanyl and tyrosyl residues; pepsin for the carboxyl group of tryptophanyl, phenylalanyl, tyrosyl, methionyl, and leucyl residues. Therefore, we propose that because of the three-dimensional structure of the native P32 molecule there are only two regions exposed to proteinase attack. Both of these regions contain one or more of the bonds specific to their respective proteinase and these bonds are proximal to one another.

Proteolysis within one of the two regions removes a peptide (or peptides) of molecular weight 8,000 to produce P32\*-I. We call it A peptide for simplicity, until further investigation can establish whether one or more than one peptides are removed. P32\*-I produced by chymotrypsin cannot be exactly the same as P32\*-I produced by trypsin. Their molecular weights and their DNA-cellulose-binding properties, however, are close enough so that they are indistinguishable on SDS gel electrophoresis or DNA-cellulose column chromatography. Similarly, proteolysis within the other region removes a peptide (or peptides) of molecular weight approximately 1,000 (B peptide) to create P32\*-II. Hydrolysis at both regions, to remove A and B peptides, produces P32\*-III. Loss of A peptide did not decrease affinity to single-stranded DNA. It did, however, increase affinity to duplex (T4) DNA in both P32\*-I and P32\*-III. Removal of B peptide resulted in decreased affinity for single-stranded DNA-cellulose. Removal of both A and B peptides, as in P32\*-III, resulted in decreased

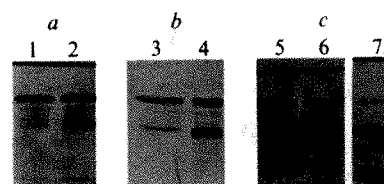


Fig. 3 Autoradiogram showing position of  $^{35}\text{S}$ -labelled P32 limited hydrolysates after electrophoresis. *a*, Column 1, untreated P32; column 2, P32 treated with  $1\text{ }\mu\text{g ml}^{-1}$  pepsin; *b*, column 3, untreated P32; column 4, P32 treated with  $1\text{ }\mu\text{g ml}^{-1}$  chymotrypsin; *c*, column 5, untreated P32; column 6, P32 treated with  $0.1\text{ }\mu\text{g ml}^{-1}$  trypsin (TPCK-treated); column 7, P32 treated with  $1\text{ }\mu\text{g ml}^{-1}$  trypsin. Digestion was in sonication buffer<sup>1</sup> at  $4^\circ\text{C}$  for 1 h. Equal volume SDS sample buffer added and samples heated in boiling water for 2 min before electrophoresis. For preparation of  $^{35}\text{S}$ -labelled P32 and electrophoresis, see Fig. 1.

affinity for single-stranded DNA but increased affinity for duplex DNA.

### Model for control of P32 activity at replication fork

Both DNA replication and recombination, in which gene 32 participates, are extremely complex reactions involving a multitude of different proteins. In such systems, it is probable that proteins function as complexes and that protein-protein interactions are the major factor in control of each component's activity.

Efforts to reconstruct the T4 DNA replication apparatus from purified components have been successful<sup>8</sup>. This system requires six T4 gene products (32, 41, 43, 44, 45, and 62) all of which are necessary for *in vivo* replication. Isolation and purification of these six has been accomplished. Because components interact synergistically with one another they probably form a complex of unique structure. This complex has a propagation reaction *in vitro* which closely mimics that found *in vivo*. All six must be present *in vitro* for extensive synthesis by strand displacement on duplex DNA templates (leading strand synthesis) and for *de novo* DNA chain initiation on single-stranded DNA templates (lagging strand synthesis), even though the individual components may not have the same activity (or any activity at all) outside the complex<sup>8</sup>.

Table 1 Column chromatography of P32 and its limited proteolysis products on DNA-cellulose

Protein	Molecular weight ( $\times 10^{-3}$ )	Expt <i>a</i> —Chromatography on denatured DNA-cellulose				Expt <i>b</i> <sup>†</sup> —Chromatography on native DNA-cellulose				
		NaCl* (M)	Adsorption		Elution		Adsorption	Elution		
			0.05	0.4	0.60	2.0		0.005 or 0.025	0.1	0.2
P32	35		+	—	—(†)	+	—	—	—	—
P32*-I	27		+	—	—(†)	+	+	—	—(†)	+
P32*-II	34		+	+	—	—	—	—	—	—
P32*-III	26		+	+	—	—	+	+	—	—

\* In buffer *b*: 20 mM Tris-HCl, pH 8.1, 1 mM  $\text{Na}_3\text{EDTA}$ , 1 mM  $\beta$ -mercaptoethanol and 10% glycerol.

† A small fraction occasionally eluted at this concentration.

‡ T4 DNA-cellulose was irradiated with ultraviolet light<sup>17</sup>. Unirradiated T4 DNA-cellulose gave similar results (J.H. and C. Brack, unpublished).

$^{35}\text{S}$ -labelled P32 ( $0.84\text{ mg}$ ,  $2.4 \times 10^5\text{ c.p.m. ml}^{-1}$ ) in buffer *b* was treated with  $1\text{ }\mu\text{g ml}^{-1}$  (freshly dissolved) enzyme at  $4^\circ\text{C}$  for 1 h. Either TPCK ( $10^{-3}\text{ M}$ ), for chymotrypsin-treated samples, or tosyl-lysyl-chloromethyl-ketone (TLCK,  $10^{-3}\text{ M}$ ), for trypsin-treated samples, was added. After 15 min more incubation to allow time for complete proteinase inhibition, solution was loaded on DNA-cellulose column. Experiment *a* combined results of four separate single-stranded calf thymus DNA-cellulose columns<sup>16</sup>, all run at the same time. Products for each column were produced by different proteases—trypsin, chymotrypsin or pepsin. Digests containing 0.05 M NaCl were applied, columns were washed with 2 ml 0.05 M NaCl and proteins eluted off with 1 ml each of following concentrations NaCl: 0.4, 0.60 and 2.0 M. Each fraction (0.5 ml) was monitored for radioactivity, and analysed on SDS gel electrophoresis. Experiment *b* combined results of chromatography of limited hydrolysis products of chymotrypsin on four separate native T4 DNA-cellulose columns (ultraviolet-irradiated<sup>17</sup>). Columns were loaded at  $5^\circ\text{C}$  or  $20^\circ\text{C}$  with digests containing either 0.005 or 0.025 M NaCl. Proteins were eluted with following concentrations NaCl: 0.1, 0.15, 0.2, 0.4 and 0.60 M. (In all four cases products with the same molecular weight behaved the same way, regardless of loading temperature or NaCl concentration.)

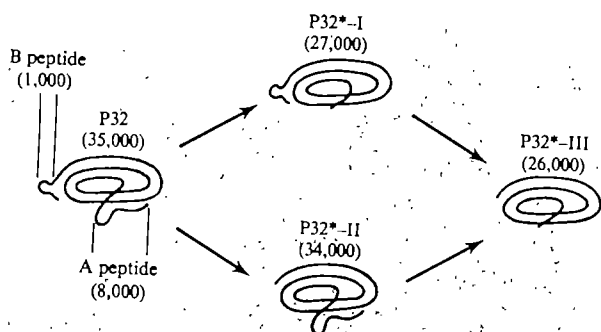


Fig. 4 Model for limited proteolysis of P32.

Two types of protein-protein interaction have been reported for P32; intra-action, that is, self-association; interaction, that is, an affinity for T4 DNA polymerase<sup>5</sup>. Self-association, as first described, was regarded as related to the cooperative binding of P32 to DNA<sup>1</sup>. Affinity for T4 DNA polymerase is related to P32 stimulation of polymerase activity<sup>6</sup>. When P32 molecules are within the replication machinery, there would undoubtedly be greater probability of increasingly complex intra- and interactions.

High susceptibility of A and B peptides to hydrolysis by various proteinases implies that these peptides are easily accessible to other proteins. Removal of either or both resulted in modification of the binding characteristics. Based on the data presented in this report, we suggest that A and B peptides are involved in protein-protein interactions and through such interactions the activity of P32, *in vivo*, is controlled.

With the removal of A peptide, affinity of P32\*-I and P32\*-III for duplex T4 DNA is greatly increased. This suggests that A peptide might be involved with the DNA-binding site in that its presence or absence greatly influences denaturing ability of the protein.

This involvement could be a direct spatial relationship to the DNA-binding site. It could also be that the interaction of A peptide with another protein leads to a conformational change in P32 resulting in a change in binding ability. It may be that, *in vivo*, A peptide limits the mode of action of P32 to that of a renaturing protein. This could have profound biological significance because the presence of too strong a denaturing protein could cause disastrous uncontrolled melting of the chromosome.

Based on this consideration we developed the following model: The role of A peptide is to localise unwinding of duplex DNA in advance of the replication fork (or within the replication complex) as shown in Fig. 5. P32, *in vivo*, acts in a renaturing or denaturing mode as regulated by A peptide and the environment in which A peptide is

placed. When P32 is outside the replication complex, A peptide exerts an inhibitory influence on denaturing, and P32 reacts in a renaturing mode. Proteolytic removal of A peptide *in vitro* produces P32\*-I with more potent denaturing activity than the original P32. In theory, the same, or even stronger, activity could be reversibly produced *in vivo* by the interaction of A peptide with the replication complex, which generates an environment in which denaturation of the duplex DNA should take place.

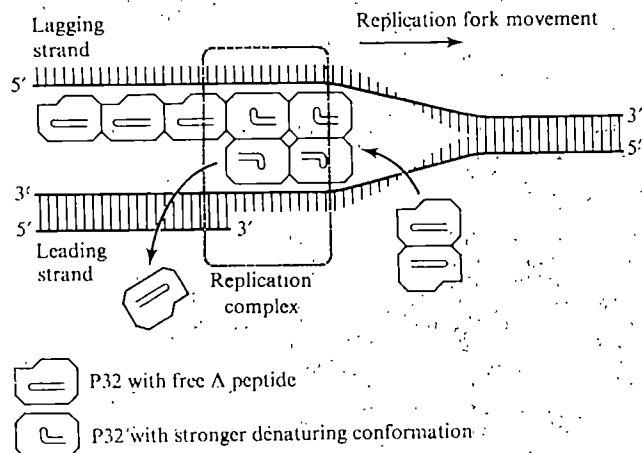
We have adopted part of a published model of self-association of P32 as follows: P32 dimer is the basic DNA binding unit in physiological conditions; the dimers insert themselves between two strands and bind to the phosphodeoxyribose backbone holding the bases in an outward position<sup>18</sup>. The advantage of this model is that with the bases held partially separated from each other, they are free to hydrogen bond with the incoming nucleotide precursors. After the replication complex (engaged in leading strand synthesis) has passed over the P32 molecules, which have been holding the duplex DNA apart, A peptide no longer interacts with the replication complex and again asserts its inhibitory influence. Thus P32 no longer prevents hydrogen bonding between the newly made leading strand and its template. P32 remains bound to the opposite strand (Fig. 5) which then acts as the template for lagging strand synthesis. P32 is required for lagging strand synthesis, and it does not fall off the single strands until lagging strand synthesis takes place.

Our data have shown that B peptide is involved in the tight binding of P32 to single-stranded DNA. There could be at least three different reasons for this: P32 has more than one DNA-binding site and B peptide contains some of them; loss of B peptide induces a conformational change unfavourable to tight binding; or B peptide is related to the cooperativity of self-association of P32.

Extensive studies of self-association by sedimentation equilibrium has led to the proposal that there are at least two types of self-association: association to dimers, which are resistant to high salt concentration and the presence of  $Mg^{2+}$  and association of these dimers to higher molecular weight aggregates in conditions of low ionic strength and high protein concentration<sup>10,11</sup>. An earlier model suggested that in physiological conditions P32 exists as dimers and association of these dimers is related to cooperative binding<sup>19</sup>. In a later model this idea was modified to suggest that binding to DNA occurs after P32 has aggregated to a higher molecular weight species<sup>11</sup>. In our more dynamic model (Fig. 5) the change of P32 to a stronger denaturing protein could include increased affinity between dimers. This would result in a contiguous binding site at the front of the replication complex, attractive to dimer-unit binding.

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Fig. 5 Model for role of P32 in DNA replication.



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# Body weight, diet and home range area in primates

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*Primates show a strong positive relationship between body weight and home range area. Dietary habits also influence home range area. Folivorous primates occupy smaller home range areas for their body weight than do frugivores and omnivores. Primates generally require smaller home range area per individual than solitary terrestrial mammals, but primates living in social groups have much larger total home range than individual solitary mammals. This trend may necessitate higher expenditures of energy in food-gathering or modifications in movement patterns.*

NUMEROUS studies during the past decade have shed light on relationships between various aspects of primate ecology<sup>1-5</sup>, but as yet there has been no quantitative summary of data on home range area of primates that takes into account the effects on spatial needs of both body size and diet. A strong positive relationship exists between body weight and home range area in lizards<sup>6</sup>, birds<sup>7,8</sup> and solitary mammals<sup>9</sup>, while McNab<sup>9</sup> showed for mammals and Schoener<sup>8</sup> for birds that feeding habits also have a predictable effect on the home range area of many species. Clutton-Brock<sup>10</sup> pointed out that rather small differences in feeding ecology may affect markedly the ranging patterns of primates. Here we analyse data on the body weight, diet and home range area of 36 primate species, and demonstrate a relationship among these variables.

The home range data in this paper are taken from diverse sources and are undoubtedly not perfectly comparable (Table 1). Most body weights were obtained by averaging adult male and adult female body weights, and thus do not take into account interspecies differences in sociometric sex ratio or the fact that many group members may be subadult. Average weights in general are probably overestimated, particularly in the case of large terrestrial omnivores like the baboon (*Papio*), in which sexual dimorphism is marked. For most species, however, sufficient data are not available, either on group composition or weight variation with age and sex, to permit calculation of more accurate average weights. In addition, rather large (for example, twofold) errors in estimating body weight will cause a shift in log weight of < 10% of the entire range of data. We believe that errors introduced by differences in techniques of home-range estimation and those resulting from inaccuracies in calculating weight are not sufficient to be critical to our general conclusions.

Diet was determined from the sources of home-range information whenever possible, supplemented by material summarised by Jolly<sup>3</sup> and Kay<sup>11</sup>. We classify as folivores those species that seem to depend on foliage, mature or immature, as their staple diet. Frugivores are primarily fruit eaters, taking only a small amount of foliage and little or no animal protein. Species eating roughly equal proportions of both foliage and fruit and little or no animal protein are classified as generalist primary consumers, and those which seem to actively seek out and probably to depend on animal protein in the diet are

classified as omnivores. Obviously there are degrees of folivory\* frugivory and omnivory and no category should be regarded as definitive; they are our best estimate of the dietary preferences of individual species. Terrestriality and arboreality, too, are points on a continuum, and assignments should not be regarded as absolute. Generally, unless a species is known to consistently travel or forage on the ground each day, we considered it arboreal.

Regressions of  $\log_{10}(\text{home range})$  on  $\log_{10}(\text{body weight})$  were fitted by the method of least squares. Significance of the regressions was determined by calculating Student's *t*-statistic for the deviation from zero of the slopes. Our criterion for significance is the 0.05 level of probability. Spearman rank correlation coefficients *r* for the untransformed data served as an additional confirmation of correlation between variables. The tests were in agreement in all cases.

## Individual home range allocation

Figure 1 shows home range area divided by the number of individuals per group ( $HR_i$ ) as a function of body weight. The species are listed in Table 1. There is a strong positive relationship between body weight and home range area in primates ( $r^2 = 0.44$ ;  $t = 5.14$ ,  $P < 0.005$ ). The slope of the regression line is 0.79, compared with 0.63 found by McNab<sup>9</sup> for temperate-zone solitary terrestrial mammals. The figure also shows each species placed in one of the four dietary categories. The more folivorous primates show a strong tendency toward a smaller  $HR_i$  than the frugivorous and omnivorous species. Eight of twelve terrestrial primates have a larger  $HR_i$  than might be expected for their weight, whereas arboreal primates show a wider scatter both above and below the regression line.

McNab divided mammals on the basis of food habits into 'hunters' (discrete particle feeders, for example, granivores, frugivores, insectivores, carnivores) and 'croppers' (browsers and grazers). Frugivorous and omnivorous primates resemble McNab's hunters in feeding habits, whereas the folivores are more like his croppers. Figure 2 shows primates grouped as hunters (frugivores and omnivores) or croppers (folivores), compared with relationships predicted by McNab for hunters and croppers. Generalist primary consumers are not considered in this comparison because they are not readily assigned to either category, and because the small sample size and weight range involved severely limits the value of any statistical treatment. The trend in this group seems, however, to resemble that among croppers more than hunters.

Both of the subgroups considered show stronger relationships between body weight and  $HR_i$  than do primates in general (for primate hunters,  $r^2 = 0.66$ , for croppers  $r^2 = 0.49$ ). The slope of the primate hunter line, 0.83, is close to that calculated for hunters by McNab, although the expected values are only about half those predicted by McNab. The slope of the line for primate croppers is 1.06, steeper than that of McNab's cropper line or the primate hunter line but not significantly different from either. Analysis of covariance of  $\log HR_i$  with feeding category and body weight for primate hunters and croppers using a randomised groups design<sup>12</sup>, shows that adjusted group means are significantly different ( $F = 69.1$ ,  $P < 0.01$ ), with croppers having substantially smaller home ranges. Primate

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croppers also tend to occupy smaller  $HR_i$  than do solitary, non-primate croppers up to  $\sim 2 \times 10^4$  g.

Values of  $HR_i$  should give some idea of the relative amount of space required to provide adequate resources for individuals of a species. What factors determine  $HR_i$ ? Clearly body size is very important; once this has been accounted for other influences become more apparent. Folivory is associated with allocation of relatively small areas per individual in arboreal primates. Most of these animals are at least partially croppers in the sense of McNab<sup>9</sup>, and are likely to have concentrated resources available. Also, the height of the tree canopy may have an especially marked effect on the size of the home range of leaf eaters. This third dimension adds to the density of resources per unit area and to the actual distance travelled in space per unit area<sup>13-15</sup>. In addition, McNab has recently suggested (personal communication) that rates of metabolism of arboreal folivorous mammals may be below those expected for mammals in general, so conceivably the energy requirements

of many folivorous primates are relatively low. All of these explanations; however, entail difficulties, some of which are discussed below.

Frugivores tend to have larger  $HR_i$  than folivores. Typical tropical forests are highly heterogeneous in species composition<sup>16-18</sup>. Fruiting trees are often widely dispersed in time and space. Thus most frugivores depend on clumped, unstable food sources, a resource pattern which has been associated with a relatively large home range area<sup>10</sup>. Also, there is some reason to suspect that frugivory is correlated with high rates of metabolism<sup>19</sup>.

Among generalist primary consumers  $HR_i$  may vary with the proportion of fruit and leaves eaten. For example, in Guanacaste, Costa Rica, where it feeds  $\sim 85\%$  of the time on foliage, *Alouatta palliata* has a small  $HR_i$  (K. Glander, personal communication) when compared with the same species on Barro Colorado Island where it spends approximately equal amounts of time feeding on foliage and fruit (K. M., personal

Table 1 Species, home range, and source of data

Species	Weight* (g)	$HR_i$ (ha)	$HR_i$ (ha)	Class†	Reference	Remarks
<b>Prosimians</b>						
<i>Galago demidovii</i>	60	0.80	0.80	AO	41	♀ only
<i>Lemur catta</i>	2,300	0.29	5.7	TP	42	
<i>L. catta</i>	2,300	0.44	7.4	TP	21	
<i>L. fulvus rufus</i>	2,370	0.10	0.88	AL	21	
<i>Lepilemur mustelinus</i>	650	0.24	0.24	AL	43	
<i>Microcebus murinus</i>	60	0.20	0.20	AO	44	♀ only
<i>Propithecus verreauxi</i>	3,800	1.8	9.0	AL	45	
<i>P. verreauxi</i>	3,800	0.48	2.4	AL	42	
<b>Cebioidea</b>						
<i>Alouatta palliata</i>	6,875	0.76	9.9	AP	K. Glander, personal communication	85% folivorous
<i>A. palliata</i>	6,875	2.8	45	AP	K. Milton, personal observation	50% folivorous
<i>A. seniculus</i>	7,250	0.38	3.2	AP	46	
<i>Ateles belzebuth</i>	5,800	14	320	AF	47	
<i>Callicebus moloch</i>	600	0.15	0.44	AO	48	
<i>Cebus capucinus</i>	3,100	6.1	86	AO	39	
<i>Saimiri oerstedii</i>	840	0.74	17	AO	49	
<i>Saguinus geoffroyi</i>	550	2.4	8	AO	50	$HR_i$ estimated from maximum linear range
<b>Cercopithecoidea</b>						
<i>Cercocebus albigena</i>	7,900	1.2	21	AF	51	
<i>C. albigena</i>	7,900	9.0	140	AF	52	
<i>Cercopithecus aethiops</i>	3,800	1.4	40	TO	53	
<i>C. mitis</i>	6,000	2.8	70	AO	R. Rudran, personal communication	Weight estimated by H. Schlichte, personal communication.
<i>C. talapoin</i>	1,130	4.0	400	AO	3	
<i>Colobus badius</i>	8,000	2.5	100	AL	10	
<i>C. guereza</i>	8,000	2.7	20	AL	10	
<i>C. guereza</i>	8,000	1.8	14	AL	54	
<i>Erythrocebus patas</i>	9,260	170	5,200	TO	55	
<i>Macaca mulatta</i>	7,300	7.9	200	TO	56	
<i>M. radiata</i>	5,140	8.9	520	TO	57	
<i>M. sinica</i>	5,130	3.0	38	TF	15	
<i>Nasalis larvatus</i>	15,100	6.5	130	AL	58	
<i>Papio anubis</i>	21,400	10	470	TO	59	
<i>P. anubis</i>	21,400	60	2,300	TO	1	Gallery forest
<i>P. cynocephalus</i>	15,100	62	2,500	TO	31	Savanna
<i>P. ursinus</i>	20,600	24	1,100	TO	1	
<i>Presbytis cristatus</i>	6,300	0.63	20	AL	60	
<i>P. entellus</i>	17,200	12	630	TL	34	Kankori site
<i>P. entellus</i>	17,200	34	640	TL	34	Orcha site
<i>P. entellus</i>	17,200	0.87	19	TL	61	Dwhar site
<i>P. entellus</i>	17,200	0.56	14	TL	15	Polonnaruwa, Ceylon, site
<i>P. johnii</i>	8,170	18	160	AL	62	
<i>P. senex</i>	5,980	3.0	12	AL	15	
<b>Hominoidea</b>						
<i>Hylobates lar</i>	5,540	11	45	AF	63	
<i>H. lar</i>	5,540	34	100	AF	20	
<i>Gorilla gorilla gorilla</i>	145,000	150	3,300	TL	64	
<i>Pan troglodytes</i>	40,700	23	1,100	TF	35	
<i>Pongo pygmaeus</i>	36,500	65	65	AP	65	♀ only
<i>Symphalangus syndactylus</i>	10,500	10	42	AP	66	

\* Body weights taken from Kay<sup>11</sup>, Napier and Napier<sup>40</sup>, or from same source as home range data.

† Indicates habitat and feeding class: A, arboreal; T, terrestrial; L, folivore; F, frugivore; P, generalist primary consumer; O, omnivore.

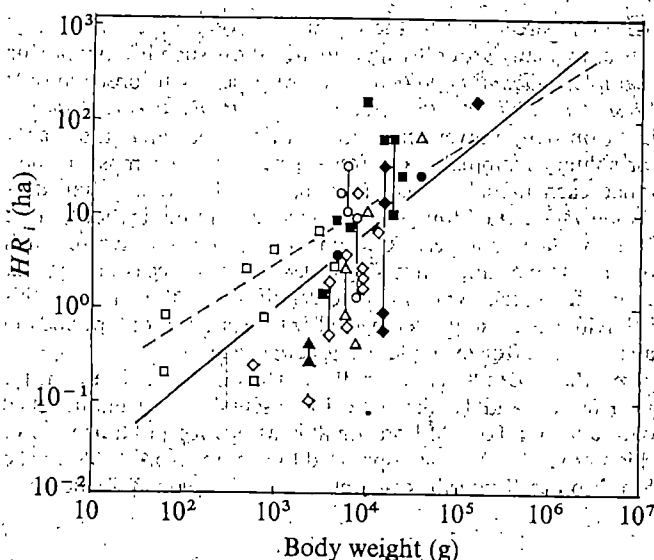


Fig. 1 Home range per individual as a function of body weight. Vertical lines connecting similar symbols indicate data from different studies of the same species. Values for the log of the mean home range for the species were substituted for these points in all calculations here and in Figs 2 and 3. Heavy solid line is least squares regression of  $\log_{10} HR_i$  on  $\log_{10}(\text{body weight})$  for primates ( $\log HR_i = 0.79 \log BW - 2.32$ ). Dashed line is regression calculated by McNab<sup>8</sup> for solitary, temperate zone mammals. Open symbols designate arboreal species; solid symbols terrestrial species.  $\diamond$ ,  $\circ$ , folivores;  $\triangle$ ,  $\bullet$ , frugivores;  $\triangle$ ,  $\bullet$ , generalist primary consumers;  $\square$ ,  $\blacksquare$ , omnivores.

observation). The siamang (*Symphalangus*), which spends ~ 50 per cent of its annual feeding time eating foliage, has a smaller home range than the gibbon (*Hylobates*), a smaller primate that seems to be more frugivorous<sup>18,20</sup>. *Lemur catta*, a generalist primary consumer that was studied at two sites, had a smaller home range where it was more frugivorous. In the study site where it ate less fruit and had a larger home range, however, it was sympatric with another lemur species, *L. fulvus rufus*, that also ate some fruit. Further, *L. catta* was much more frugivorous than *L. fulvus rufus* and had a much larger  $HR_i$  in the habitat where the two species were sympatric, even though they have about the same body weight<sup>21</sup>.

Arboreal omnivores generally occupy a large  $HR_i$  for their body weight, as large, in fact, as terrestrial omnivores. This suggests that arboreality *per se* does not reduce the area required to provide adequate nutritional resources for omnivores. Arboreal omnivores probably depend more heavily on insects in the diet than the larger, terrestrial omnivores, and may, therefore, occupy a slightly higher trophic level than their terrestrial counterparts. Nevertheless, solitary arboreal squirrels, which are also discrete particle feeders (but are certainly primarily herbivorous), have  $HR_i$  comparable to the primate  $HR_i$  of this group<sup>22,23</sup>.

Terrestrial primates as a group tend to have an  $HR_i$  somewhat larger than expected for their body weight. Some of these species live in arid regions that probably have lower densities of resources per given area<sup>2</sup>. The patas monkey (*Erythrocebus patas*) is an extreme case and it has a very large  $HR_i$ . Home range data are not available for two other arid country species, the hamadryas baboon (*Papio hamadryas*) and the gelada (*Theropithecus gelada*), but information on daily range indicates that they also cover very large home range areas<sup>2</sup>. Except for these arid land forms, it is not clear that terrestriality in itself is correlated with large  $HR_i$ . Terrestrial folivores do have larger  $HR_i$  values than arboreal folivores, but the differences may arise simply from their larger size.

Primate hunters and croppers generally have lower values of  $HR_i$  than predicted for temperate zone solitary mammals of corresponding feeding habits. The small individual home ranges may reflect: (1), the fact that some hunters may some-

times graze or browse; (2), the high productivity of many tropical areas compared to mesic temperate zone habitats; (3), the additional resource space provided by the depth of the forest canopy; or (4), the greater efficiency of utilisation of area as a result of being social. All of these factors may contribute in particular cases. Certainly some frugivores, and very probably some terrestrial omnivores, eat enough leaves or grass to make a substantial impact on their resource area requirements. The year-round high productivity of many tropical regions may be important, but much of this productivity may be unavailable as animal food<sup>18,24-26</sup>. The idea that the greater vertical extent of the resource space for arboreal species should reduce the  $HR_i$  required is attractive *a priori*, but it is not clear why this should apply to folivores only. Some of the possible effects of sociality are discussed below.

We have not tried to take into account the effect on home range area of overlap between conspecific groups, although one might expect that  $HR_i$  as well as total home range would be larger in species with greater overlap. The little data available suggest this may be true, since most species with non-overlapping ranges (for example, *Callicebus moloch*, *Symphalangus syndactylus*, *Colobus guereza*, *Propithecus verreauxi*), occupy home range areas smaller than or equal to the predicted values.

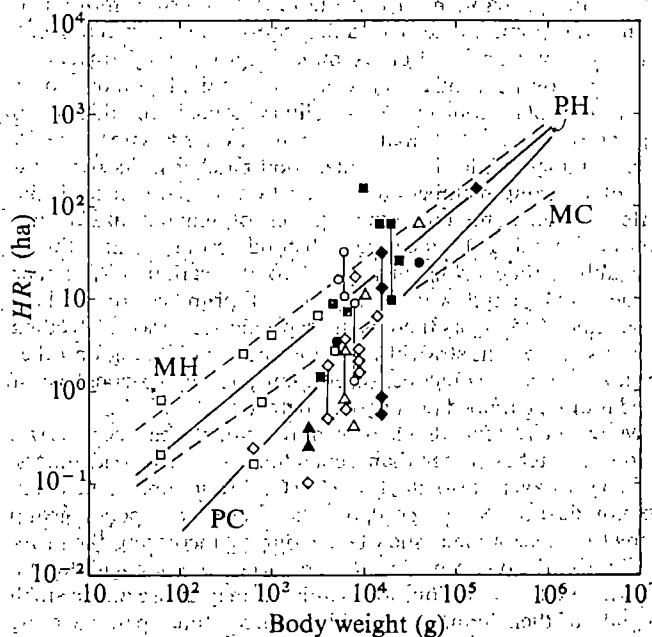
### Total home range

Figure 3 shows total group home range ( $HR_t$ ) as a function of body weight. The slope of the regression is significant ( $r^2 = 0.56$ ;  $t = 6.54$ ,  $P < 0.005$ ). Much of the deviation from the regression in Fig. 3 may be due to variation in group size, since there is a strong correlation between group size and  $HR_t$  ( $\rho = 0.67$ ;  $P < 0.0001$ ) irrespective of body weight.

Broad patterns of variation in total group home range are generally similar to those noted for  $HR_i$ . Both folivores and generalist primary consumers tend to have smaller  $HR_t$  than frugivores and omnivores. To a greater extent than  $HR_i$ ,  $HR_t$  of terrestrial species tends to be higher than expected.

Perhaps the most interesting aspect of Fig. 3 is that  $HR_t$  for many group-living primates is far larger than the home range of solitary animals of similar body weight, although solitary

Fig. 2 Influence of diet on  $HR_i$ . Heavy solid lines are regressions of  $\log_{10} HR_i$  on  $\log_{10}(\text{body weight})$  for frugivores and omnivores (primate hunters, PH;  $\log HR_i = 0.83 \log BW - 2.17$ ) and folivores (primate croppers, PC;  $\log HR_i = 1.06 \log BW - 3.66$ ) separately. Generalist primary consumers are excluded from the calculations. Dashed lines are regressions calculated by McNab<sup>8</sup> for solitary, temperate zone hunters and croppers (MH, hunters; MC, croppers). Species symbols as in Fig. 1.



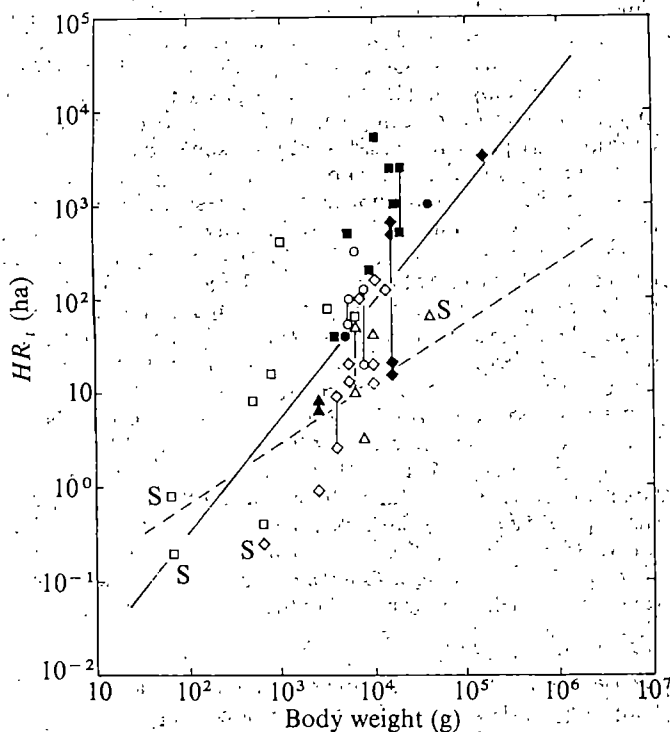


Fig. 3 Total home range per group as a function of body weight. Symbols as in Fig. 1. S indicates solitary foragers. Regression equation for primates is  $\log HR = 1.23 \log BW - 2.86$ .

primates occupy home ranges similar to those of other mammals. If, within a given time interval, the primate group covers the same proportion of its home range using the same movement patterns as a solitary mammal, the individual primate may have to expend much more energy in travel than solitary mammals of similar size. How can social primates afford or avoid the higher cost of obtaining their resources? We can suggest three possibilities which are not mutually exclusive and the second and third may be related. First, it is possible that locomotion is a negligible portion of the energy budget. This seems unlikely in view of what is known of the metabolic cost of locomotion<sup>27</sup>. Second, it may be that foraging as a group increases food-finding efficiency enough to compensate for increased travel costs. Undoubtedly energy required for foraging is reduced by a shared pool of traditional knowledge about resource timing and location and travel routes<sup>28-30</sup>. Third, and probably most important, the pattern of movement within the home range may differ consistently between social and solitary animals. For example, a group may traverse a large area over a broad front while each individual moves over a fairly straight course<sup>31,32</sup>. A solitary animal would have to zig-zag back and forth many times to cover the same area. A social animal can feed on smaller food patches in its path and cue other group members to the location of rich patches if the latter are encountered. Some species of iguanid lizards use conspecific cuing for locating rich food resources, and this is probably a time-saving method of food location<sup>33</sup>. A similar model might apply within a group of primates, on a short time scale. As long as there is intraspecific tolerance of feeding at the same patch, straight line movement with cuing on other group members would be an efficient strategy, particularly if food is often clumped in fairly rich patches.

Many social animals seem to move directly between known resource patches rather than depending on chance encounters. Solitary foragers also do this (G. G. Montgomery, personal communication), but perhaps, as suggested above, group knowledge encourages such behaviour by increasing the probability that resource patches will be accurately recognised and remembered. In addition, social animals may utilise only a small portion of their home range within a given time span, covering

the entire area only in the course of weeks or even months. Certainly many group-living primates show such behaviour (for example, *Alouatta palliata* (K. M., personal observation); *Presbytis entellus*<sup>34</sup>; *Pan troglodytes*<sup>35</sup>; *Papio cynocephalus*<sup>31</sup>). Data on daily movements within their home range are scanty for solitary mammals. Red foxes generally cover most of their range each night<sup>36</sup>, while raccoons<sup>37</sup>, rabbits<sup>38</sup>, and anteaters (*Tamandua* (G. G. Montgomery, personal communication)) do so in a few days to a week. Certain social primates traverse much of their home range in a single day, at least occasionally, but except for species which defend relatively small territories, this behaviour is usually interspersed with relatively long periods in a small area (for example, *Cebus capucinus*<sup>39</sup>).

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# letters to nature

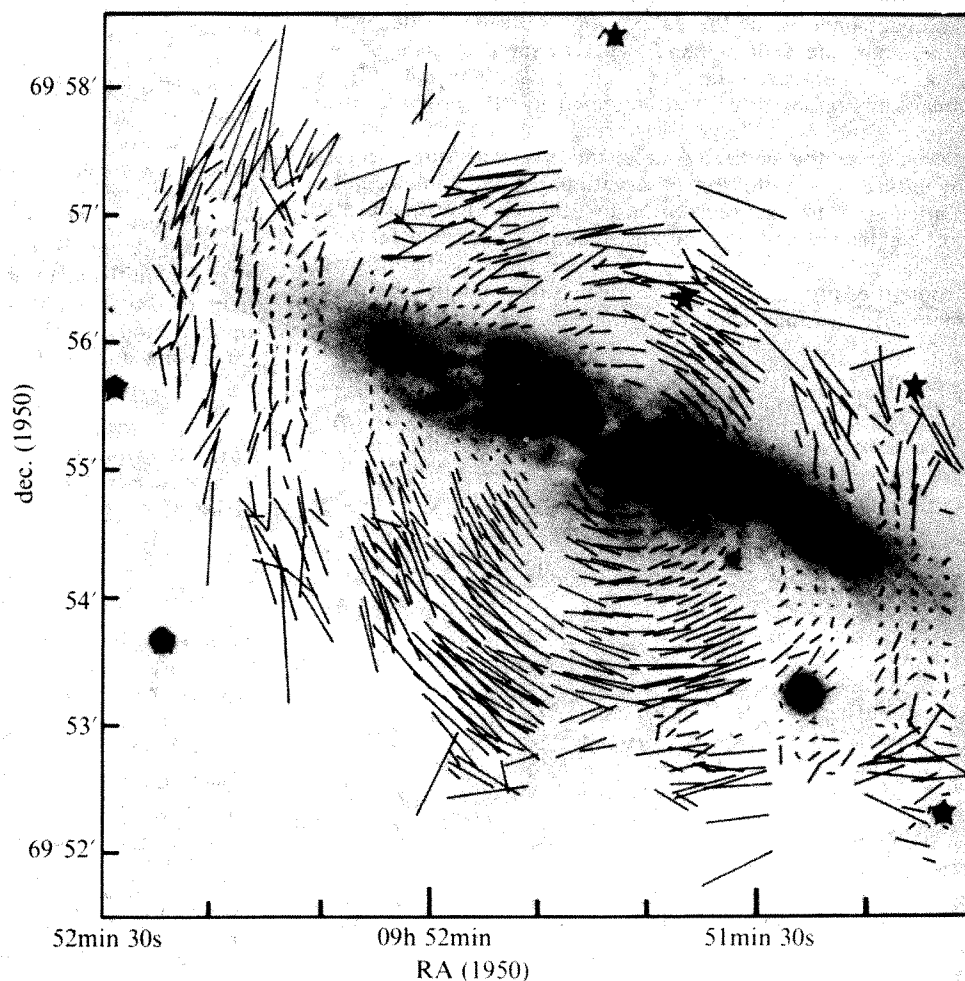
## New map of the optical polarisation of galaxy M82

M82 is the nearest galaxy evidently containing a strong central source of energy, which is possibly similar to the nucleus of a Seyfert galaxy, and this seems to have given rise to an unusual explosive event<sup>1</sup>. The appearance of M82 is that of a flattened irregular system seen nearly edge-on, and if a distinct nucleus exists, it is highly obscured<sup>2</sup> at optical wavelengths. Compact radio features are, however, present, and complex resolved radio and infrared sources and bright visible H II regions occupy an area  $\sim 12'' \times 35''$  in the centre of the galaxy. The outer parts of the galaxy show linear optical polarisation, discovered by Elvius<sup>3</sup>. This is thought to arise from reflection in an extensive halo of dust particles of the light of the bright nucleus (or nuclear region) and of the galactic disk (for a recent discussion, see ref. 4). Consequently, observation of the polarisation can give information about the position and luminosity of the energetic nuclear region, and about the scattering medium. Outstanding questions about M82 involve the existence and nature of a compact nucleus and the morphological and evolutionary state of the whole galaxy.

The observations reported here were obtained with the 1-m telescope of the Wise Observatory, Israel, during the period February 27–March 15, 1975. The polarimetric technique is derived from that of Ohman<sup>5</sup>, but uses a rotating achromatic half-wave plate, and a Wollaston prism to separate orthogonally polarised beams, which are recorded simultaneously and remain in a fixed relationship to the detector. The technique and the computer programmes used in the analysis will be discussed elsewhere. The detector was an electronographic camera<sup>6</sup> on loan from the Royal Greenwich Observatory to the Wise Observatory. The waveband used was B of the UBV system.

The results of the observations are shown in Fig. 1. Each measurement is indicated by a line parallel to the plane of the electric vector and proportional in length to the degree of polarisation, centred on the point observed. Each determination is made over an area  $\sim 8'' \times 8''$ . The map has a superimposed photograph of M82 to illustrate the relationship of the polarisation structure to the bright part of the galaxy. The photograph is from a blue plate taken on the Isaac Newton telescope by Dr S. V. M. Clube. The eight stars indicated were used for astrometric purposes.

These observations show the polarisation at more than ten times the total number of points previously observed, with a spatial resolution three to five times finer. The



**Fig. 1** The linear optical polarisation of M82 in the B waveband. ★ represent fiducial stars, ♦ the centre of symmetry, —, 10% polarisation.



**Table 1** Comparison between our measurements and published values for polarised stars

Star	Tabulated value		This work	
	$P(\%)$	$\theta(\text{deg})$	$P(\%)$	$\theta(\text{deg})$
HD 43384	$2.75 \pm 0.05$	$170 \pm 1$	$2.3 \pm 0.4$	$172 \pm 5$
HD 122945	$0.1 \pm 0.07$	$56 \pm 18$	$0.3 \pm 0.4$	$69 \pm 20$
HD 155528	$4.6 \pm 0.09$	$93 \pm 1$	$4.3 \pm 0.4$	$90 \pm 3$
HD 80083	$0.13 \pm 0.09$	$140 \pm 17$	$1.4 \pm 0.4$	$128 \pm 10$

results are complete over a large area of the galaxy, but as we are carrying out further work which may eventually serve to extend and improve the data, we refrain from tabulating the extensive numerical results at this stage.

The accuracy of the observations has been checked by comparison with the previous work<sup>3,7,8</sup> on M82, and by observations of individual stars of known polarisation<sup>6</sup>. The comparison between our measurements and published values for the stars is given in Table 1. From these results, it was not considered necessary to make any corrections for instrumental effects. Also from these results and from the comparison with previous work on M82 and from our internal consistency, the standard error over most of the map, for a typical point with 15% polarisation was estimated as 2% in degree of polarisation, and 3° in position angle. These errors are comparable with previous work. The weaker alignment of the polarisation vectors seen near the edge of the map probably arises from the reduced contrast of the galaxy against the sky background, introducing a noise component. A further interpretation of these results will be given elsewhere, but a preliminary note of the more outstanding features is made here.

The completion of the two-dimensional polarisation study provides data for the construction of improved models of M82. For example, the fairly clear division of the area into regions of high and low polarisation represents a separation in depth, at least near the centre of the map. The edge-on view of the galaxy shows the near side of the disk along the central band, whereas on each side of the disk an unobscured line of sight passes through highly polarised regions directly illuminated by the bright central source, and with large scattering angles. We hope to characterise the central source and the reflecting medium by fitting models to the observational data. Some smaller contribution to the polarisation by other mechanisms is also to be expected and may be apparent in one or two features of the galactic disk in Fig. 1; these most probably arise from the properties of the disk rather than the halo material, and may show that there is some magnetic field

alignment in this irregular object. The centrally symmetric pattern enables a position to be found for the central source of radiation, following Solinger and Markert<sup>4</sup>. The position obtained is indicated in Fig. 1 and on a larger scale in Fig. 2, superimposed on optical and infrared contour maps, and with other interesting positions marked. The centre of symmetry may be influenced by emission from the disk as well as the nucleus of the galaxy (Solinger and Markert assume the disk is symmetrical), and it is not at present clear what difference this will make. The position obtained was, however, found to be consistent, within the indicated error, when different areas of the map were used in its derivation, and it is quite independent of previous polarisation studies.

Some previous writers have been at pains to emphasise that various central features in M82 were almost coincident, but we would point out that there is no detailed correspondence between them, such as would be expected if a true nucleus were present. The most intense radio component (cross-hatched in Fig. 2) has no special position in the infrared source, which itself is only near to the centre of symmetry. The optically brightest spot, which is sometimes assumed to be the centre in dynamical studies, is close to the centre of the infrared source but not to the other features.

The lack of an identified singular nucleus does not, however, preclude the possibility that M82 has some resemblance to a Seyfert galaxy. A compact nucleus may be present, but still unidentified, and even if no such nucleus exists, an interesting possibility arises from McCrea's theory of the Seyfert phenomenon<sup>10</sup>, which he ascribes to supernova events at the rate of perhaps one every 10 yr, in a limited volume. (No direct triggering of the events is called for, but star formation may be stimulated by previous outbursts.) In the case of M82, referring to Fig. 2, the active region of the galaxy may be ~300 pc in diameter, about ten times the size of a Seyfert nucleus, and the process would be much modified; however, it seems particularly appropriate for a galaxy such as M82, containing as it does a large amount of interstellar matter and regions of star formation (H II regions) within the 300 pc central active domain. A galaxy of this type, if we had a face-on view, might not qualify as a Seyfert from its spectral characteristics, but could represent the connecting link between Seyferts and normal galaxies.

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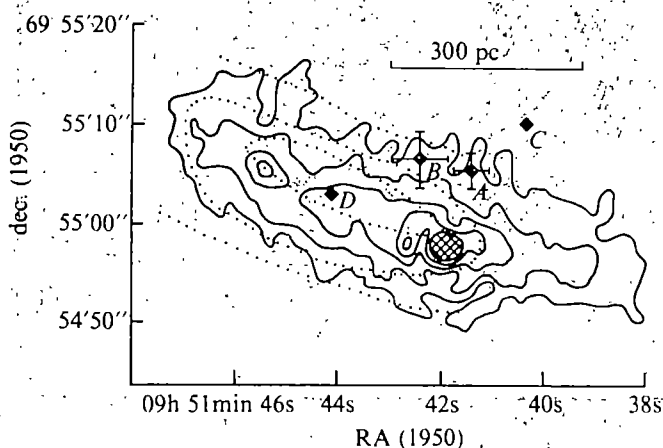
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**Fig. 2** The central region of M82 showing 5 GHz contours<sup>11</sup> and 10  $\mu\text{m}$  contours (dotted)<sup>12</sup>. The positions marked are: (A) the centre of symmetry of the optical polarisation (this paper) (B) the centre of symmetry<sup>4</sup> (C) the radiant of the filamentary structure<sup>13</sup> (D) the optical bright spot sometimes assumed to be the nucleus.



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## Variations of the infrared polarisation of VY Canis Majoris

A REMARKABLE change in the infrared polarisation of VY Canis Majoris has been observed in 1974 and 1975. This star was found, in 1970, to have a large and peculiar polarisation<sup>1</sup>. Further observations carried out in 1971 and 1972 confirm the existence of a polarisation peak at  $1.6 \mu\text{m}$  and a rapid rotation of the position angle of polarisation from visible to infrared wavelengths<sup>2-3</sup> (see Fig. 1). The polarisation declines rapidly with wavelength from ultra-violet to infrared, having a dip near  $1 \mu\text{m}$ , and rising further in the infrared, with peak at  $\lambda = 1.6 \mu\text{m}$ . Corresponding to the rapid decrease in the polarisation, the position angle rotates by about  $90^\circ$ . Our latest observations show that the hump at  $1.6 \mu\text{m}$  has disappeared.

Our original results suggested that the polarisation could be resolved into two components, visible and infrared polarisations, and the visible polarisation has since been

Fig. 1 The wavelength dependence of the degree of polarisation and the position angle for the observations in 1969 and 1972. +, Serkowski 1969; ●, Shawl 1969; ○, our results 1970; ○, our results 1971; △, Forbes 1971; × Dyck et al. 1971; □, Serkowski, 1972.

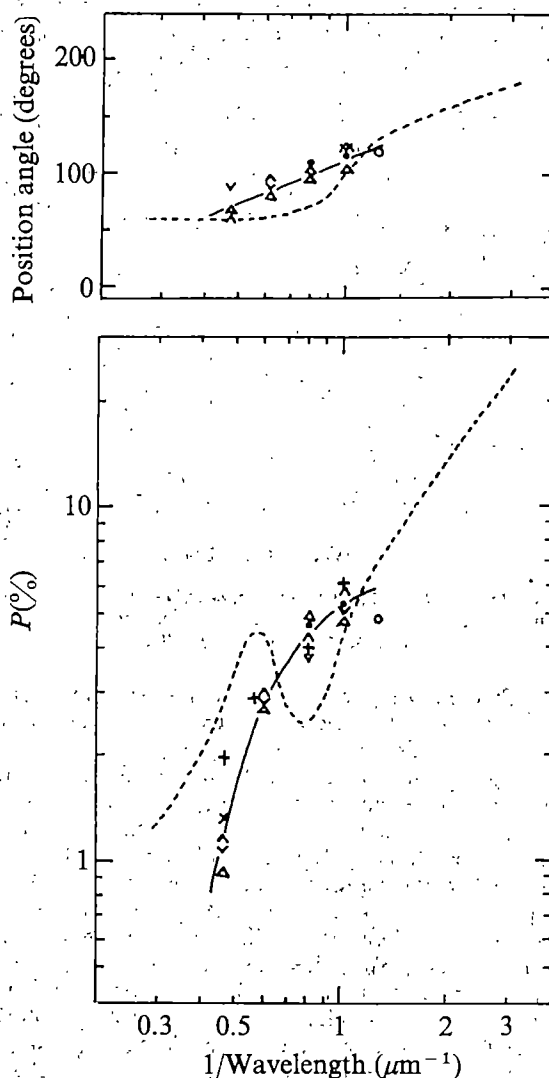
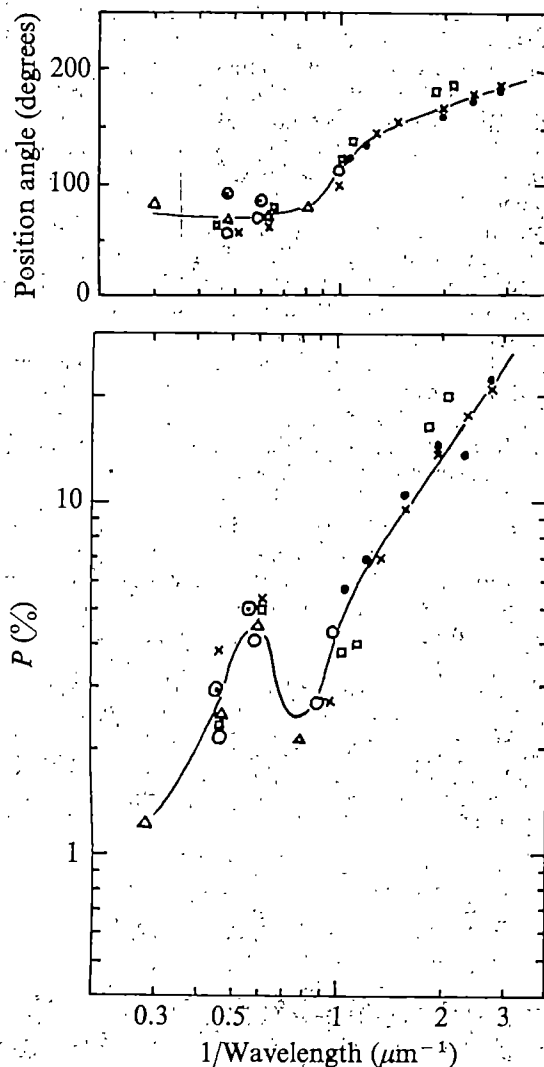


Fig. 2 The same as in Fig. 1 but for 1974 and 1975 (+, January, 1974; ×, ●, △, V, Λ, ○ February–April, 1975).

shown to originate mostly from a reflection nebula<sup>4</sup>. High resolution photographic observations have shown that the nebula is highly polarised ( $\leq 70\%$ )<sup>5</sup>. Such a high polarisation can be explained only in terms of Rayleigh-like scattering by very small grains of radii  $< 0.1 \mu\text{m}$ . These small particles, however, would not be capable of producing the infrared polarisation.

The infrared polarisation would have to be attributed to scattering by much larger grains, of radii  $\sim 1 \mu\text{m}$ , concentrated in a fairly dense cloud around the central star. The existence of the polarisation indicates that the distribution of this dust cloud (infrared nebula) is not spherically symmetric, but very asymmetric—probably disk shaped<sup>6,7</sup>.

We repeated the observation in 1974 and 1975, and found some remarkable changes in the infrared region. The observations were done with the same procedure as before<sup>1</sup>, but using a 1-m infrared telescope equipped with a secondary mirror wobbling chopper. The new results are summarised in Table 1 and shown in Fig. 2, in which the average values of the old results are also shown as a dashed line.

Surprisingly, the peak at  $1.6 \mu\text{m}$  has disappeared, the infrared polarisation is smoothly connected with the visible polarisation. Since the observation was regrettably interrupted by the construction of the telescope, when and how the change occurred is not known precisely.

The photometric observations, however, have not shown any significant change in this same period, as shown in

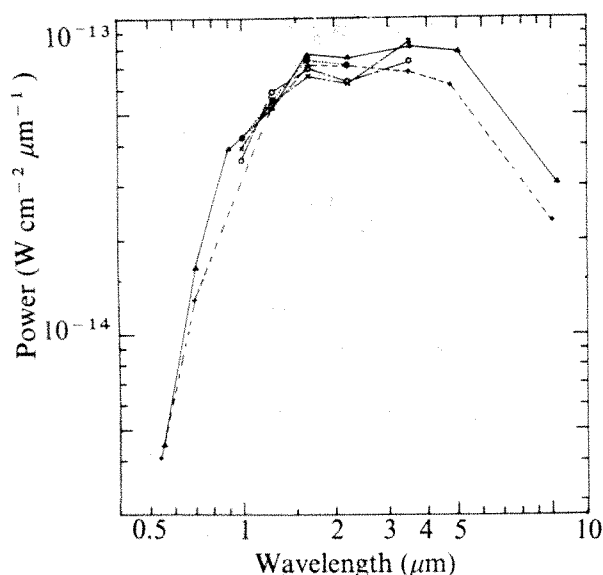


Fig. 3 Photometric observations between 1969 and 1975. (+, Hyland *et al.*, 1969;  $\Delta$ , Low *et al.* 1970; O, our results, 1971;  $\times$ , our results, 1974;  $\odot$ , our results, 1975).

Fig. 3. This implies that neither a drastic change in the stellar emission, nor a global change in the configuration of the dust clouds could have occurred.

The change could have been limited to a very localised region with extremely high polarisation. It might have been caused by a dust cloud ejected from the star. An alternative possibility is that the infrared nebula consists of many fragments, and an outer cloud which lets light out through a gap. The motion of the fragments inside the cloud would then bring different fragments to view, through the gap, and the observed polarisation would change. In this case any gross change in appearance would not necessarily be associated with high speed motion of matter. This is more suitable for explaining a change of the polarisation in visible regions<sup>5,7</sup>, which arises from an extremely large scale reflection nebula.

It has been reported that IRC+10216 showed a periodic variation in infrared polarisation, which correlated with an anti-phase intensity variation<sup>8</sup>. It is interesting in the case of VY Canis Majoris to see whether the change is periodic or not, and what change occurs in optical polarisation as well as in the circular polarisation which had been detected by Serkowski<sup>9</sup> and Gehrels<sup>10</sup>.

Table 1 Polarimetric observations of VY Canis Majoris

Date	$\lambda(\mu\text{m})$	$P(\%)$	Position angle
1974 January 20	1.02	$5.7 \pm 0.4$	—
	1.25	$4.0 \pm 0.3$	—
	1.65	$3.0 \pm 0.2$	—
	2.25	$1.9 \pm 0.1$	—
1975 February 3 February 8 March 1 March 8 March 12 April 9	2.25	$1.1 \pm 0.1$	$58 \pm 2$
	1.02	$4.5 \pm 0.2$	$126 \pm 1$
	1.25	$4.0 \pm 0.1$	$108 \pm 1$
	1.02	$4.1 \pm 0.4$	$108 \pm 2$
	1.25	$4.4 \pm 0.1$	$104 \pm 1$
	1.65	$2.2 \pm 0.1$	$87 \pm 1$
	2.25	$0.8 \pm 0.2$	$56 \pm 4$
	1.02	$4.7 \pm 0.2$	$117 \pm 1$
	1.25	$3.9 \pm 0.2$	$108 \pm 1$
	1.65	$2.4 \pm 0.1$	$80 \pm 1$
	2.25	$0.7 \pm 0.1$	$66 \pm 1$
	1.02	$5.4 \pm 0.2$	$118 \pm 1$
March 12	1.25	$4.4 \pm 0.2$	$100 \pm 1$
	1.65	$2.3 \pm 0.1$	$89 \pm 2$
	2.25	$1.1 \pm 0.1$	$53 \pm 3$
	0.9	$5.1 \pm 0.1$	$119 \pm 1$

For a definite conclusion, much more extensive and continuous observations are necessary in linear and circular polarimetry and photometry from the ultraviolet to infrared regions, as well as photographic studies.

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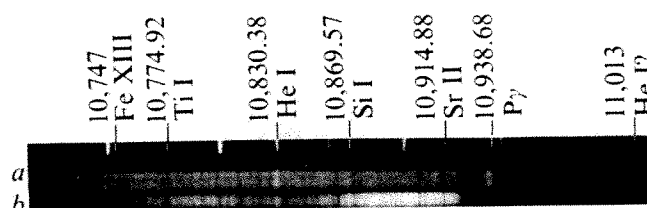
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## Fe XIII line in R Aquarii

GREGORY and Seaquist<sup>1</sup> have detected radio emission from the interesting object R Aquarii<sup>2</sup>. In the course of reducing plates of this star made in 1970 and 1971 in the course of my He I,  $\lambda = 10,830 \text{ \AA}$  spectral survey, I found the emission line of the forbidden coronal transition of Fe XIII at  $\lambda = 10,747 \text{ \AA}$ . This line ( $^3P_1 - ^3P_0$ ) is often accompanied by the  $^3P_2 - ^3P_1$  line at  $\lambda = 10,798 \text{ \AA}$  at high densities ( $N_e > 10^9$ ). The emission line  $\lambda = 10,747 \text{ \AA}$  is weak, but clearly seen on two spectra (Fig. 1), with intensity of  $\sim 100 \text{ m\AA}$ . The spectrum also shows strong emission lines of He I at  $10,830 \text{ \AA}$  and  $\text{P}\gamma$  as well as a line at  $11,013 \text{ \AA}$  which may be He I  $3^1S - 6^1P$ . I have examined an old plate made by Merrill in 1948 for traces of the coronal lines at  $5,303 \text{ \AA}$  and  $6,374 \text{ \AA}$  (Fe XIV and Fe X) and found them absent. G. Wallerstein (personal communication) has kindly examined his plates from 1970–71 and finds the  $6,374\text{-\AA}$  line definitely absent. If the lines are excited by the  $2,400\text{-K}$  radiation field of the star, the  $6,374\text{-\AA}$  and  $5,303\text{-\AA}$  lines should be much fainter than the  $10,747\text{-\AA}$ , because of their higher excitation energy.

Some idea of the physical conditions in the corona of R Aquarii may be found from the intensity of the line ( $100 \text{ m\AA}$ ) and the absence of other coronal lines. We assume a corona (or part of the nebula) at  $10^6 \text{ K}$  (coronal ionisation—ionisation at  $10^6 \text{ K}$  and  $N_e < 10^{10}$ —is density independent). If collisional excitation were responsible, we would require  $N_e^2 V \sim 3 \times 10^{45} \text{ cm}^{-3}$ . In that case the line at  $10,798 \text{ \AA}$  would

Fig. 1 Two image tube spectrograms of R Aquarii. *a*, September 1968, phase 0.01 (maximum light); *b*, July 1970, phase 0.79, with identified lines and wavelengths ( $\text{\AA}$ ) marked. The Fe XIII line is at extreme left, near the  $10,749\text{-\AA}$  Si I absorption line. Strong He I and  $\text{P}\gamma$  emission is also seen. There are a number of other possible emission lines in the spectrum, but none is identified and they may just be windows in the continuum.



be at least as strong as that at 10,747 Å, however, and the coronal lines in the visible would be excited by the thermal electrons and be much stronger relative to the continuum there. If the coronal density is low, we will still have the ionisation corresponding to the kinetic temperature, but the ions will be excited by photospheric radiation at a rate

$$F_{12} = \frac{1}{w} \frac{g_2}{g} \frac{A_{21}}{\exp(h\nu/kT) - 1} = \frac{0.05}{w} \text{ s}^{-1}$$

for  $T_e = 2,000$  K with  $w$  the dilution factor. Integrating this over a uniform nebula, we find

$$R_{\text{cor}} N_{\text{Fe}} = 2.1 \times 10^{14} \text{ cm}^{-2}$$

or

$$R_{\text{cor}} N_e = 2 \times 10^{18} \text{ for } N_e/N_{\text{Fe}} = 10^4$$

The scale height of such a corona, if distributed barometrically, would be 100 AU, but of course the gravity could not keep it from escaping and other factors must play a role. For  $\lambda = 5,303$  Å,  $A_{21}$  is six times greater, but the exponential term is  $10^{5.6}$  greater, so the emission is  $6 \times 10^4$  less and the line cannot be seen, even though the continuum is lower by 6 mag than the 1.1 μm value.

The thermal radio flux from this corona is, at  $\lambda = 3.5$  cm,  $1.44 \times 10^{-15} R_{\text{cor}} \text{ mJy}$ . If the source size is  $< 1''$ ,  $R_{\text{cor}} < 4 \times 10^{15}$ , and a flux of 5.5 mJy is obtained; for  $T = 2 \times 10^6$  K (also possible for Fe XIII), we have 8 mJy, in rough agreement with the flux measured by Gregory and Seaquist.

We conclude that R Aquarii is surrounded, *inter alia*, by a corona at  $T \approx 10^6$  K and  $R \sim 100$  AU, which produces the observed iron line and may be the source of the radio emission observed.

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*Note added in proof:* A spectrogram obtained on November 23, 1975, on the rise ( $M \sim 9$ ) after an exceptionally deep minimum, showed no Fe XIII emission, but extremely intense P Cygni-type He emission, as well as Pγ emission.

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## Pulsars and magnetic monopoles

THE report<sup>1</sup> of the possible existence of magnetic monopoles makes some speculations about their astronomical role worthwhile. Pulsars possess magnetic fields, possibly the highest in the Universe<sup>2</sup>, and it thus seems natural to speculate how these magnetic fields might affect monopoles and how the latter, in turn, could influence the behaviour of the pulsars.

For concreteness, we adopt Schwinger's dyon picture of magnetic monopoles<sup>3</sup>. The magnetic charge  $g$  is then  $6 \times 137 \times e$  and the monopole mass  $M$  gives  $Mc^2 = 400$  GeV. One very important fact is that such a monopole acquires  $\sim 0.25$  MeV by travelling 1 cm in a magnetic field of  $\sim 1$  gauss.

Let us estimate the absorption cross section of a pulsar for a monopole of energy  $\epsilon$ . Assuming that the pulsar field is that of a static dipole, we find that the perpendicular momentum acquired,  $\Delta p$ , by a monopole passing at a distance  $r$  is given approximately by the relationship ( $c$ : velocity of light)

$$\Delta p = g^2 M^2 / \epsilon r^2 c \quad (1)$$

where  $M \approx B_0 R^3$ , is the magnetic moment of the pulsar with maximum surface field  $B_0$  and radius  $R$ . Consider a monopole moving along a path which intersects field lines that do not

curve too much along their approach towards the stellar surface (that is, the collision takes place within not too wide a cone around the magnetic axis). This monopole will be appreciably deflected and thus may be channelled into the pulsar polar region, provided  $\Delta p \gtrsim p$ , or, equivalently, if  $r \lesssim \sqrt{(gM/\epsilon)}$ . This leads to a capture cross section  $\sigma$  (up to a numerical factor of less than unity arising from the geometry)

$$\frac{\sigma}{\sigma_{\text{geom}}} \approx \frac{gM}{\epsilon R^2} \approx 3 \times 10^{16} \frac{RB_0}{\epsilon} \quad (2)$$

where  $R$  is the radius in km,  $\epsilon$  the energy in MeV, and  $B_0$  is in units of  $10^{12}$  gauss.

It remains to estimate  $\epsilon$ . Passage through random magnetic fields in interstellar space probably leads to a substantial acceleration of the monopoles. A further energy gain could be acquired near the pulsar by means of its non-dipolar magnetic field. Such a process would be rather efficient in the vicinity of normal stars where spiralling magnetic field lines carried by the stellar wind extend over large distances: for example, one can estimate that the interplanetary space around the Sun with  $B = 10^{-5}$ – $10^{-6}$  gauss,  $l = 10^8$  km, would provide  $Bl \approx 10^3$ – $10^6$  gauss cm and a pre-acceleration to  $10^{14}$  eV before the actual impact on the dipole field takes place. In contrast, the corresponding mechanism for pulsars is probably much less important, because of the lack of a 'pulsar wind', and because the region where static non-potential magnetic field configurations can occur is limited to within the light cylinder, where  $Bl$  is appreciably smaller than in the interplanetary space.

It is probably safe to take an upper limit of  $\sim 10^{11}$  eV for  $\epsilon$ . For the pulsar case, this would make  $\sigma/\sigma_{\text{geom}} \sim 10^6$ – $10^7$ , making the capture cross section equal to the cross section of the light cylinder, beyond which, anyhow, one would not extrapolate the static formula, or the simple picture of the capture given here. On the other hand for normal stars  $B_0 R$  is substantially lower, and  $\sigma/\sigma_{\text{geom}} \sim 1$ , indicating that in contrast to pulsars, monopoles would arrive at the stellar surface more or less uniformly.

One can thus conclude that pulsars might have a capture cross section for monopoles  $\sim 10^{18}$ – $10^{20}$  cm<sup>2</sup>. Impinging monopoles would be funnelled into the polar regions. Their terminal energy at impact can easily be estimated from

$$\int_{\infty}^R gB(r) dr \approx gB_0 R$$

to be  $\geq 10^{22}$  eV, if we take  $B_0 \sim 10^{12}$  gauss,  $R \sim 10^6$  cm.

Along their way to the pulsar surface the monopoles suffer energy losses due to curvature radiation<sup>4</sup>. If this were the only energy loss, their energy balance would be described by the relationship

$$\epsilon/c = Bg - A\epsilon^4; A = \frac{2}{3}(g^2/\rho^2)(Mc^2)^{-4} \quad (3)$$

where  $\rho$  is the radius of curvature of the trajectory. The resulting saturation energy  $\epsilon_{\text{max}}$  is given by the equation

$$\epsilon_{\text{max}}/Mc^2 \approx (B\rho^2/g)^{1/4} \quad (4)$$

which leads to  $\epsilon_{\text{max}} \approx 10^{21}$ – $10^{22}$  eV (probably an underestimate) if we take for  $\rho$  the radius of curvature of the last open field line, or  $\rho \approx$  radius of the light cylinder  $\approx 10^8$ – $10^{10}$  cm. The value of  $\epsilon_{\text{max}}$  found here is sufficiently large that the value of the terminal energy we found earlier is not appreciably affected. We note, by the way, that the ratio of the radiation reaction force,  $2g^4 B^2 / 3M^2 c^4$ , to the accelerating force  $Bg$  is  $10^{-61} B$  (gauss), which is so small that the ordinary radiation damping can be neglected.

Although a monopole crossing the pulsar magnetosphere will also be affected by the electric field in it<sup>4</sup>, its gyroradius  $r$  in



an electric field  $E$ , which is given by the equation

$$r = \varepsilon/gE \quad (5)$$

will be sufficiently large not to affect appreciably the monopole trajectory.

Let us now investigate whether the pulsar magnetic field could be substantially extinguished by the accretion of monopoles. For this to happen, each pulsar hemisphere has to collect  $N$  monopoles such that  $NgR \simeq B_0 R^2$ . The number of required monopoles is then

$$N \simeq B_0 R^2 / g \simeq 2 \times 10^{30} \quad (6)$$

If we take the pulsar lifetime to be  $\sim 10^{15}$  s, the cosmic flux of monopoles must be  $\lesssim 10^{-4} \text{ s}^{-1} \text{ cm}^{-2}$ , as the extinction of the pulsar magnetic fields seems not to have taken place. This upper limit is 6 orders of magnitude higher than that set already by similar considerations about the Earth's magnetic field<sup>5,6</sup> and 9 orders of magnitude higher than that set by the lack of more than one event found in cosmic ray work<sup>1</sup>.

It thus seems that no useful conclusions regarding the flux of cosmic magnetic monopoles can be drawn from the persistence of pulsar magnetic fields. The situation is not, however, quite as simple as this, because of the high terminal energy of the monopoles. In fact, near the pulsar surface, the accelerating field imparts to the monopole an energy equal to the monopole rest mass energy over a distance of  $\lesssim 10^{-6}$  cm. In a collision with a nucleus, there will be enough energy in the centre-of-mass system to produce a pair of monopoles, if the  $\gamma$  of the monopole equals or exceeds  $\gamma_0 = 2M/m_p \simeq 1,000$  ( $m_p$ : proton mass), or  $\varepsilon \simeq 10^{15}$  eV. We see that there will thus be ample energy to create monopole pairs. The wrongly charged member of the pair will be hurled back into extra-pulsar space with an energy  $> 10^{20}$  eV (compare the mechanisms for pair production in the Sturrock<sup>7</sup> and Ruderman-Sutherland<sup>4</sup> theories of pulsar emission) while its companion will be further accelerated towards the pulsar. If the conditions

$$\gamma_0 Mc^2 / gB < \lambda_{\text{loss}} \text{ and } \lambda_{\text{pair}} \ll l^* \quad (7)$$

are satisfied, where  $\lambda_{\text{loss}}$  is the mean free path for energy losses not associated with pair creation,  $\lambda_{\text{pair}}$  is the pair creation cross section mean free path and  $l^*$  the width of the region with a density sufficiently high for copious pair creation to occur, an avalanche of new monopoles may be generated. The energy loss of a monopole in ordinary matter has been estimated<sup>8</sup> to be  $10^{12} \text{ eV g}^{-1} \text{ cm}^2$ . A layer of width  $\sim 10^{-1}$  cm in the outer crust with a density  $\sim 10^4 \text{ g cm}^{-3}$  would be a suitable environment for the avalanche production of monopoles. If we estimate the number of monopoles  $N_*$  produced in the avalanche to satisfy the relationship

$$N_* Mc^2 \gamma_0 = \varepsilon \quad (8)$$

and use  $\varepsilon \simeq 10^{21}$  eV, we find  $N_* \simeq 10^6$ , and the persistence of the pulsar magnetic fields would lead, for pulsars such as PSR 1933 or PSR0527 with a relatively short lifetime (and a large  $B_0$  in the case of PSR0527), to quite a low upper limit on the monopole flux, which may even be as stringent as the one given in ref. 1.

It is, of course, impossible to estimate  $N_*$  in any reliable way, as long as data on monopole pair-production cross sections are lacking. Nevertheless, in view of the availability in the pulsar crust of a relatively large region where the monopoles undergo frequent collisions while still, on the average, gaining energy from the magnetic field, a very substantial multiplication can be expected to occur.

To summarise our main conclusions: first, the persistence of pulsar magnetic fields may possibly set a more stringent limit on the cosmic flux of magnetic monopoles than the presently available one. The actual value of the limit can only be given,

once the pair-production cross section is known. Second, pulsars may act as copious sources of very high energy (secondary) monopoles (if monopoles really exist). It would be of interest to look for correlations between monopole events and pulsar positions, if monopole events are observed in any significant number.

The annihilation of a pair of oppositely charged monopoles will surely have an extremely low probability. Even if there were the  $10^{30}$  monopoles needed to extinguish the pulsar magnetic field, their density would only be  $\sim 10^{12} \text{ cm}^{-3}$ , and the annihilation mean free path would be many orders of magnitude larger than the pulsar radius. If the monopoles are also electrically charged, as in the dyon model<sup>3</sup>, their accumulation would induce an additional electric dipole field which would, however, be  $\lesssim 10^7 \text{ V cm}^{-1}$  so that its effect could be negligible compared to the already existing magnetospheric field.

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## No anisotropy in angular diameter-redshift relationship

Using data for the brightest cluster galaxies, we here consider whether any anisotropy exists in the angular diameter-redshift relationship. This is in response to the finding of Rubin *et al.*<sup>1</sup>, who described a 'curious' distribution of radial velocities for ScI type galaxies having apparent magnitudes  $m$  in the range  $14 < m < 15$ . For two distinct regions of sky, the mean radial velocity for these galaxies differs by  $\sim 1,400 \text{ km s}^{-1}$ ; the corresponding mean apparent magnitude differs by  $\sim 0$  mag, compared with the difference of 0.5 mag expected on the basis of this velocity difference. They tentatively concluded that the Hubble parameter  $H$  is not isotropic.

Le Denmat and Vigier<sup>2</sup> examined the distribution of  $HM$  (see ref. 1) for type I supernovae<sup>3</sup> that have occurred in a wide variety of galaxies, and found a highly significant anisotropy that agrees with that described by Rubin *et al.* We note, however, that the supernova apparent magnitudes given in ref. 3, and used by Le Denmat and Vigier<sup>2</sup>, seem to be mostly post-maximum values and therefore only set lower limits on  $HM$ : little weight can therefore be placed on this result. Jaakkola *et al.*<sup>4</sup> have also found an anisotropy in  $HM$  for compact galaxies with absorption spectra, but they further found that the degree of anisotropy decreases with increasing distance.

If the effect found by Rubin *et al.*<sup>1</sup> is truly cosmological in origin, and is not the result of selection effects, then it should manifest itself in other 'cosmological' relationships, such as the angular diameter-redshift relationship. Rubin *et al.*<sup>1</sup> did not find, with certainty, any difference in the mean angular diameter of the ScI galaxies in their sample, but they described the values they used as being of low weight.

Data obtained<sup>5</sup> from measurement of 48" Schmidt plates form a reasonably homogeneous sample of isophotal angular

diameters for E galaxies having redshift  $z$  in the range 0.004–0.085. Less extensive and less homogeneous are the data measured from 200'' plates, also tabulated by Sandage<sup>5</sup>. We neglect both the 'K-like' correction, that transforms angular diameters to the rest frame of each galaxy (this correction amounts to  $\sim 6\%$  at most for the 48'' data), and the 'seeing' correction ( $\lesssim 1''$ ), which is small by comparison with the smallest angular diameter in the 48'' sample ( $\sim 10''$ ). For the smallest angular diameters in the 200'' sample, the net effect of these two corrections is zero<sup>5</sup>.

Isophotal angular diameters,  $\theta$ , are proportional to  $z^{-1}$  for  $q_0 = +1$ , which is a sufficiently good approximation for our range of  $z$  values. For each galaxy we compute the quantity  $A = \log \theta + \log cz$ , which is analogous to the  $HM$  of Rubin *et al.*<sup>1</sup>.  $A$ , which for low redshifts is a measure of the linear dimensions of galaxies for uniform  $H$ , is not correlated with  $z$  for either sample. Writing  $\langle A \rangle$  for the mean value of  $A$  in each region, we should expect

$$\langle A \rangle_{II} - \langle A \rangle_I = \langle HM \rangle_{II} - \langle HM \rangle_I = \sim 0.1$$

(see ref. 1) if the anisotropy<sup>1</sup> is indeed 'cosmological'. If the alleged anisotropy is not, however, cosmological then we should expect that the difference in the  $\langle A \rangle$  should merely reflect the sampling error, that is  $\langle A \rangle_{II} - \langle A \rangle_I = 0$ .

For the 48'' sample,  $\langle A \rangle_{II} - \langle A \rangle_I = 0.024 \pm 0.030$ , a difference that is not statistically significant (probability  $P = 0.44$ ). In addition there is no tendency for high or low values of  $A$  to be correlated with the regions I and II of Rubin *et al.*<sup>1</sup> ( $P = 0.52$ ). Even if we suppose that the above difference in  $\langle A \rangle$  arises entirely from a difference in  $H$  (that is, we assume that the brightest cluster galaxies in the 48'' sample are both standard candles and standard rods) then  $H_{II}/H_I = 0.95^{+0.55}_{-0.35}$  from the isophotal angular diameter-redshift relationship. This ratio is not significantly different from unity, and in any case is in the opposite sense to that claimed previously<sup>1,2,4</sup>. We note also that no significant difference between regions I and II is found when metric, rather than isophotal, angular diameters are considered. Furthermore, no anisotropy exists in regions of sky other than those delineated by Rubin *et al.*<sup>1</sup>, nor does anisotropy become apparent when various redshift intervals are considered.

Unfortunately sufficiently accurate and homogeneous apparent magnitude values were not available for the 48'' sample so we could not test whether the  $\langle HM \rangle$  for this sample differs significantly between regions I and II. For the smaller and less homogeneous 200'' sample, however, (for which  $\langle A \rangle_{II} - \langle A \rangle_I = 0.051 \pm 0.037$ ;  $P = 0.20$ ),  $\langle HM \rangle_{II} - \langle HM \rangle_I = 0.034 \pm 0.050$  which does not differ significantly from zero ( $P = 0.50$ ). It should be noted, however, that Jaakkola *et al.*<sup>4</sup> have found that the anisotropy is apparently present in their sample of brightest cluster galaxies.

If the anisotropy apparent in the  $HM$  values of ScI galaxies and compact galaxies with absorption spectra is indeed cosmological, then it could be masked in the angular diameter-redshift relationship by the intrinsic dispersion in the linear dimensions of E galaxies. After removal of dispersion from measuring errors, Sandage<sup>5</sup> estimates that the linear diameters of brightest cluster galaxies have a dispersion of  $\sim \pm 20\%$ ; the corresponding dispersion in  $\log \theta$  is  $\approx 0.1$ . Neglecting errors in redshift values, the dispersion in  $A$  arising from this dispersion is  $\sim 0.1$ , which is precisely the magnitude of the difference  $\langle A \rangle_{II} - \langle A \rangle_I$  expected on the basis of the difference  $\langle HM \rangle_{II} - \langle HM \rangle_I$ .

On the other hand, it has similarly been argued<sup>6</sup> that the dispersion in the absolute magnitudes of the galaxies considered could, in certain circumstances, give rise to an apparent anisotropy in  $HM$ . Although Rubin *et al.*<sup>1</sup> regarded the possibility of large scale inhomogeneities unlikely as a means of accounting for the anisotropy, Sandage and Tammann<sup>6</sup> have suggested that inhomogeneities on a scale  $\sim 100$  Mpc compounded with the narrow range of  $m$  values considered in ref. 1,

could explain the Rubin *et al.* result. The equality of the  $\langle m \rangle$  values for regions I and II would then merely reflect the narrowness of the apparent magnitude range chosen by comparison with the dispersion in the absolute magnitudes of ScI galaxies (0.4 mag, ref. 7). Jaakkola *et al.*<sup>4</sup>, however, found that the anisotropy does seem to exist ( $P = 0.015$ ) for compact absorption galaxies with  $13 < m < 18$ .

A further consideration in discussions of effects of this kind should, however, be the homogeneity of the data used. Sandage<sup>8</sup> has argued that much of the dispersion in the absolute magnitudes of brightest cluster galaxies arises from differing observational errors (that is, different observing sites and observing conditions) and clearly this argument would also apply to the absolute magnitude dispersions of other types of galaxy. Similarly, the effect of personal observing habits, as discussed by Penston and Rowan-Robinson<sup>9</sup> in connection with the alleged anisotropic redshift distribution of quasars, could also give rise to systematic observational errors.

Data used in previous examinations<sup>1,2,4</sup> of the Hubble anisotropy have been taken from catalogues in which apparent magnitude values have been obtained from a number of sources and it would be difficult to argue that these data are sufficiently homogeneous in the sense discussed above. Thus any anisotropy in the apparent magnitude-redshift relationship should be regarded as 'not proven' until analysis of a sufficiently homogeneous sample of galaxies shows otherwise.

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## Present trends in the Earth's magnetic field

We outline here some of the implications of the latest in a series of models of the Earth's magnetic field derived in association with the World Magnetic Charts published by the British Hydrographic Office. The model, details of which are published elsewhere<sup>1</sup>, is defined by spherical harmonic coefficients representing the internal part of the magnetic field (to twelfth order and degree; 168 coefficients), its secular variation (to eighth order and degree; 80 coefficients), and its secular acceleration (the 26 most significant coefficients) at epoch 1975.0. Because of the greatly improved distribution, accuracy and quantity of data incorporated in the analysis, we believe that this model, particularly with regard to the secular variation, provides a significantly better representation of the Earth's magnetic field than any previous model. It is of interest to compare some of the parameters derived from this model with the trends shown by its predecessors.

To a first approximation (and an extremely good one) the Earth's magnetic field can be represented by that of a uniformly magnetised sphere—the so-called 'centred dipole' model. The orientation of the dipole axis ( $78.7^\circ\text{N}$ ,  $289.5^\circ\text{E}$ ) and its motion

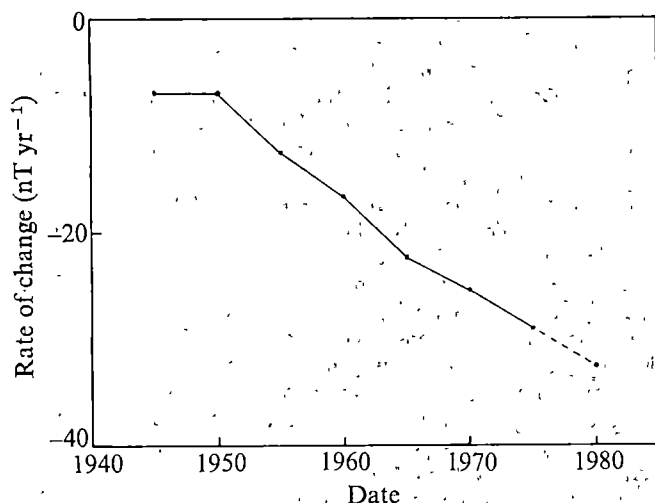


Fig. 1 Rate of decrease of dipole moment. The point for 1970 is from an unpublished model based on observatory data; that for 1980 is obtained by updating the 1975 model using its time-dependent coefficients, and is included to show the trend they imply.

( $0.00^\circ \text{ yr}^{-1} \text{ N}$ ,  $-0.06^\circ \text{ yr}^{-1} \text{ E}$ ) follow closely the trends found by Malin<sup>2</sup>, although they suggest that the westward motion of the axis is slowing down. It is usual to measure the strength of the dipole by  $MR^{-3}$ , where  $M$  denotes the dipole moment and  $R$  the mean radius of the Earth. For the present model, this quantity is 30,701 nT, and is decreasing by 28.8 nT  $\text{yr}^{-1}$ . This rate of decrease is the largest yet found, but is in good agreement with the rather speculative trend noted earlier (see refs 3 and 4, and Fig. 1). The recent points in Fig. 1 are in excellent agreement with the predictions of Malin and Clark<sup>4</sup> which were based on the assumption of a linear change in the decrease of dipole moment, and there seems little reason to revise their estimate that, if present trends were to continue, the field would reverse in about 2230 AD.

A better approximation to the main field is obtained by displacing the dipole from the Earth's centre, without changing its magnitude and direction, to the magnetic centre—the 'eccentric dipole' model. The displacement vector is (474 km,  $19.8^\circ \text{ N}$ ,  $148.0^\circ \text{ E}$ ) and its rate of change ( $2.2 \text{ km yr}^{-1}$ ,  $0.26^\circ \text{ yr}^{-1} \text{ N}$ ,  $-0.05^\circ \text{ yr}^{-1} \text{ E}$ ). The distance of the magnetic centre from the centre of the Earth continues to increase at a remarkably constant rate; there is no support to the suggestion<sup>5</sup> that

the magnetic centre is moving in an ellipse. The rate of northward drift continues to increase slowly, but, more interestingly, the westward drift, previously seen to be slowing down<sup>4</sup>, has now virtually stopped.

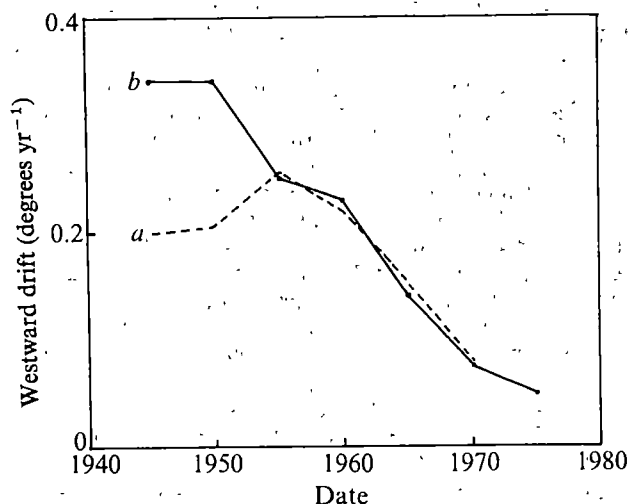
The magnetic dip poles are those points on the Earth's surface at which the lines of force are vertical. The model indicates that the north magnetic dip pole is located on Bathurst Island at ( $76.1^\circ \text{ N}$ ,  $259.5^\circ \text{ E}$ ) moving eastwards at  $5 \text{ km yr}^{-1}$ . The south pole is at ( $65.6^\circ \text{ S}$ ,  $139.6^\circ \text{ E}$ ), about 120 km off the coast of Adelie Land, and is moving approximately north-eastwards, towards New Zealand, at  $5.5 \text{ km yr}^{-1}$ . The exact positions and movements of such features as the dip poles may be significantly affected by nearby anomalies, and are better determined by local surveys than by a global model. The positions and movements are, however, entirely consistent with the survey data obtained near the north magnetic pole<sup>6</sup>. Recent survey data are not available for the vicinity of the south magnetic pole, but the declination measures from the nearby observatory of Dumont d'Urville are in good agreement with those expected to be associated with the pole. It is interesting to note that both of the dip poles show an eastward rather than a westward motion.

The evidence seems to indicate that the westward drift of the magnetic field, which has been going on at least since the time of Halley<sup>7</sup>, has recently slowed down and may even have stopped. A numerical estimate of the westward drift is obtained by taking a global mean of longitudinal movements of certain features of the Earth's magnetic field. The best method of taking this mean, and the most appropriate features to consider are still open to discussion. For example, should one consider total intensity,  $B$ , or vertical intensity,  $Z$ ; pattern at the surface of the Earth or at the core-mantle boundary; the whole field or only the non-dipole part of the field? The results obtained from several of these methods are given in Table 1.

Table 1 Westward drift (degrees  $\text{yr}^{-1}$ )

	1975 (ref. 1)	1942.5–1962.5 (ref. 2)
Centred dipole	0.06	0.09
Eccentric dipole	0.05	0.29
Richmond's method (ref. 8):		
Whole field ( $Z$ )	0.09	0.13
Whole field ( $B$ )	0.10	0.15
Non-dipole field ( $Z$ )	0.13	0.21
Non-dipole field ( $B$ )	0.12	0.17
Yukutake's method (ref. 9):		
Whole field	0.07	0.14
Non-dipole field	0.10	0.24

Fig. 2 Westward drift in degrees  $\text{yr}^{-1}$ . *a*, From length-of-day data; *b*, for the eccentric dipole. Point for 1970 *b* as in Fig. 1.



Although the different estimates vary considerably, they all agree that the westward drift is much smaller in 1975 than it was during the interval 1942.5–1962.5, by a factor of two on average. The main reason for this is the diminution in the secular variation of the  $h_2^1$  term, which has for many years been the major contributor to westward drift, and accounted for the large apparent difference between the drift rates of the dipole and non-dipole parts of the field noted in the past. A more reasonable division might have been into  $h_2^1$  and non- $h_2^1$  parts of the field. Traditionally, the westward drift has been interpreted as the difference between the rotation rates of the outer layers of the core (where the field originates) and the mantle. If that were so, the recent reduction in westward drift would imply either a transfer of angular momentum from the mantle to the core—which should be accompanied by an increase in the length of the day—or a redistribution of angular momentum within the core. Following Vestine and Kahle<sup>10</sup> we have adopted the former interpretation, calculated the westward drift that would account for the observed length of day (Fig. 2*a*), and compared this with the observed westward drift of the eccentric dipole (ref. 4 and Fig. 2*b*). The mean ordinate of Fig. 2*a* is arbitrary, and

its scale can be adjusted by altering the thickness of the layer of outer core to which angular momentum is transferred. We have chosen values that bring the recent portions of curves *a* and *b* close together, but to achieve that the thickness of the core layer had to be reduced to the improbably low value of 100 km. Even so, there seems to be little correlation between the two curves, and we conclude that a direct relationship between the westward drift and the length of day has yet to be established.

Regardless of the physical interpretation of westward drift, it provides a simple concept that accounts for much of the secular change of the non-dipole part of the field (though its deficiencies in this respect have been emphasised by James<sup>11</sup>). A better approximation to the secular change is given by a simple rotation of the non-dipole part of the Earth's magnetic field about an axis other than the geographical axis<sup>12</sup>. For the 1975 model, the rotation rate is  $0.145^\circ \text{yr}^{-1}$  in a clockwise direction (when viewed from above) about an axis with its pole at  $(76.1^\circ \text{N}, 273.0^\circ \text{E})$ . The close proximity of this pole of rotation to the dip pole is attributable to chance, as may be seen by examining earlier positions for the pole of rotation<sup>13</sup>. This latest result confirms and extends the trends discussed by Malin and Saunders<sup>13</sup>.

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## Possible late Precambrian subduction zone in South West Africa

CONTINENTAL margin plate tectonism during the late Precambrian in southern and central South West Africa and western Botswana is strongly suggested by the geographical distribution, stratigraphy, composition and evolutionary characteristics of the pre-Damara, pre-Nama formations (1,350–900 Myr old) occurring north of the Namaqua Tectonic Province<sup>1</sup>. If the distribution of these units is considered it is apparent that a unique pattern exists (Fig. 1). The data for the generalised geological map of Fig. 1 are taken from various sources<sup>2–9</sup> and show: first, the distribution has the form of a prominent and extensive curvilinear feature or 'arc'. Second, the arc separates the undeformed late Precambrian deposits (Nama Group<sup>10</sup>) of the stable platform area—the Kalahari Craton or Plate—from the highly deformed and metamorphosed deposits of the late Precambrian Damara orogenic belt, suggesting that the arc occupies a marginal cratonic position, and third, the trend of the arc and arc structures (such as major faulting) parallels that of the Damaran structural trends, suggesting a possible relationship between the geotectonic development of the arc and the Damaran Orogeny (see Fig. 1).

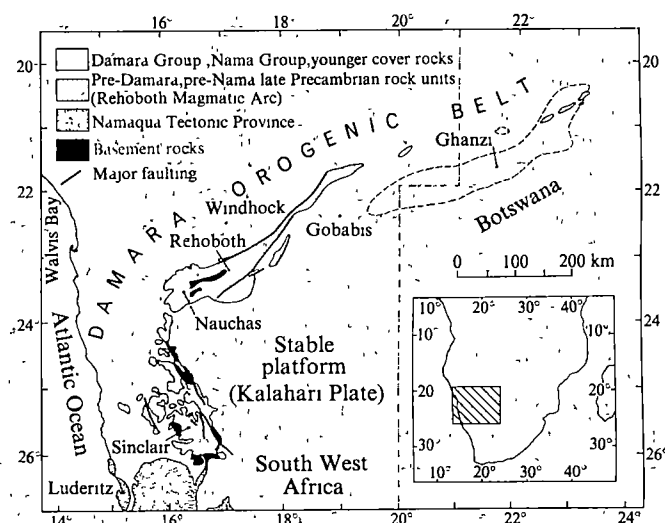


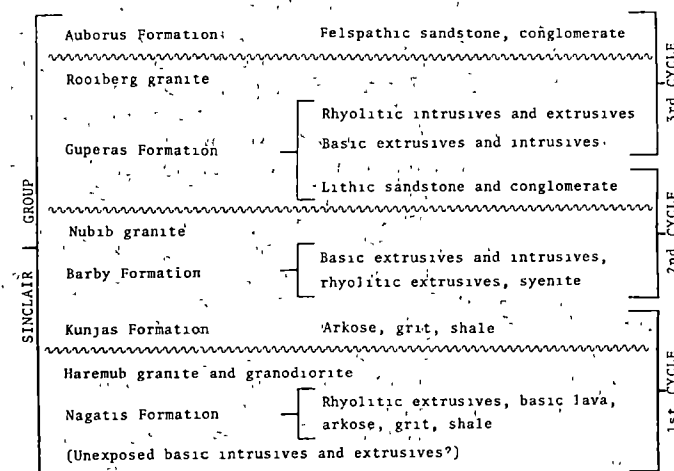
Fig. 1 Distribution of the pre-Damara and pre-Nama late Precambrian formations in southern and central South West Africa and western Botswana.

The various units comprising this late Precambrian arc consist essentially of highly variable sequences of basic, intermediate and felsic lava and volcanoclastics, as well as thick sedimentary deposits which are frequently of obvious local provenance. The sequences were extruded on to and deposited on an older sialic basement of unknown age and were intruded by large amounts of comagmatic high level granitic, rhyolitic and basic to intermediate material. The name Rehoboth Magmatic Arc has been proposed for this volcano-plutonic belt<sup>6</sup>.

The best exposed and most intensively studied part of the belt is the southern portion which is the type area for the Sinclair Group<sup>9</sup>, the geological succession of which is summarised in Fig. 2. The evolution of the Sinclair Group proceeded within three major cycles. The basis for this cyclic subdivision is seen in a repeated pattern of development. The generation and emplacement of basic to intermediate magma into and on to the continental crust, followed by the generation and emplacement of felsic magma into high crustal levels, subsequent vertical tectonics and production of local fault trough basins, followed by erosion of the earlier igneous units and rapid deposition of immature clastic debris into the local basins. The cycle ends with a period of relatively little faulting and tilting.

Basic and intermediate volcanism is shown by extensive piles of calc-alkaline and, to a lesser extent, tholeiitic,

Fig. 2 Geological succession and evolutionary cycles of the late Precambrian Sinclair Group, South West Africa.





flows, building up locally to thicknesses of 8,000 m within individual cycles. The calc-alkaline lavas are characteristically highly porphyritic (plagioclase feldspar and/or pyroxene) and range from 'normal' basalts, basaltic-andesites and andesites to highly potassic varieties having all the compositional and modal characteristics of shoshonites<sup>11,12</sup>. Basic intrusives are comagmatic and represented by tholeiitic gabbros often displaying igneous layering, by basaltic dykes and by bodies of calc-alkaline porphyritic basalt, gabbro, diorite, monzonite and syenite.

Felsic units are high level granite plutons and batholiths, rhyolitic dykes, plugs, domes, flows and volcanoclastics. A distinct compositional break exists between the felsic and basic igneous rocks of the Sinclair Group, and the available evidence indicates that the felsic magmas represented the first products of melting of predominantly lower crustal material<sup>9</sup>. Heat for the fusion process was evidently supplied by the enormous volumes of basic magma transported through the crust, resulting in the basic, followed by felsic, succession, of magmatic events found in the various evolutionary cycles of the group. The apparent lack of an extensive basic magmatic event initiating the first cycle could conceivably be attributable to emplacement of magma into a relatively cool crust and consequent rapid and early chilling, with only minor volumes of basic magma reaching the surface. Furthermore, the emplacement and deposition of the younger units has largely obliterated evidence for the early stages of the first cycle.

Cessation of magmatic activity within each cycle was followed by periods of intense erosion of local volcanic piles and exposed plutons with the deposition of immature clastic debris into local basins which began forming toward the latter stages of the felsic magmatic events. The basins were the result of vertical movement along extensive faults paralleling the general trend of the Rehoboth Magmatic Arc, which in the Sinclair Group type area, is approximately north-north-west.

Other parts of the arc provide evidence for similar evolutionary patterns to that of the Sinclair Group<sup>3,4,6-8</sup> although never as complete or as well preserved; the ENE trending sector has been involved to varying degrees in the younger Damara tectonism, with thrusting and shearing to the SSE. Sequences are typically of limited lateral extent with interfingering relationships, consisting of thick piles of basic, intermediate and felsic volcanics, and immature clastics; comagmatic intrusion is extensive. Granodiorite and quartz diorite (as opposed to highly siliceous granites) are, however, more common within the Nauchas-Rehoboth sector of the arc than in the Sinclair Group type area.

I regard the characteristics of the Rehoboth Magmatic Arc and its constituent units as reasonable evidence for the presence of a subduction zone active during late Precambrian times beneath the western and north-north-western margin of the Kalahari Plate. In terms of modern plate tectonics a requisite for continental margin tectonics is the presence of a stable plate, and the Kalahari Plate has certainly remained essentially undeformed since Sinclair Group times (since ~ 1,350 Myr), inferring that the craton had acquired its present stable and consolidated nature before the development of the Rehoboth Magmatic Arc.

The well-defined curvilinear shape, situated at the edge of a highly stable continental plate, thick piles of tholeiitic, calc-alkaline and shoshonitic extrusives and comagmatic intrusives, voluminous 'granite' batholiths and rhyolitic lava, intrusives and volcanoclastics, episodic or pulsing development, vertical tectonics paralleling the trend of the arc, and rapidly deposited immature clastic debris of local provenance, are all characteristics of the Rehoboth Magmatic Arc that are common to young continental margin plate tectonic regimes<sup>13,16</sup>.

It is suggested, therefore, that the presence of this volcano-plutonic arc can be used to define roughly the position

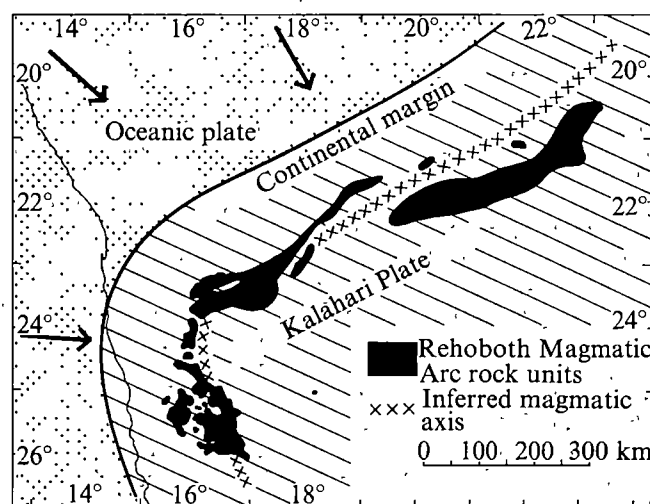


Fig. 3 Suggested reconstruction of the geotectonic setting that may have prevailed during the evolution of the late Precambrian Rehoboth Magmatic Arc, superimposed on a sketch map showing the present-day distribution of the Arc units. Arrows indicate the approximate directions of plate movement.

of the margin of the Kalahari Plate during immediately pre-Damara times. The distance between the continental margin and the axis of a volcano-plutonic arc developed above a subduction zone at a continental margin has been estimated to be in the order of  $225 \pm 50$  km (ref. 17). To the west of the Sinclair area (the southern sector of the Rehoboth Magmatic Arc (Fig. 1)) younger Damara rocks are encountered 180 km to the west along the present coastal regions of South West Africa. It is probable, therefore, that these younger rocks delineate fairly accurately the position of the continental slope regions of the Kalahari Plate during the late Precambrian. In the east-north-east trending sector of the arc, thrusting of the Damara Group units (during the Damara Orogeny) to the south-south-east over the magmatic arc units has occurred and the original position of the continental margin is a matter of speculation. A suggested reconstruction of the Kalahari Plate margin and prevalent geotectonic setting during evolution of the late Precambrian Rehoboth Magmatic Arc is presented in Fig. 3.

The model proposed here for the evolution of the Rehoboth Magmatic Arc may have far-reaching implications for the development of the Damara Belt, because it implies an oceanic crust during pre-Damara times in the area that is now overlain by the Damara Group. Oceanic crust in the area during the late Precambrian would be consistent with a paratectonic or collision origin for the Damara, as suggested for all pan-African orogenic belts by Burke and Dewey<sup>18</sup>. Such a model can, therefore, reconcile the apparently conflicting evidence for the origin of the Rehoboth Magmatic Arc by continental margin subduction, with the supposed ensialic character<sup>19</sup> of the following Damara orogenic event.

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## A novel beryllsilicate phase containing 3-coordinate beryllium ( $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$ )

BERYLLSILICATES are normally framework structures, with Be in tetrahedral coordination, similar to Si (ref. 1), and the structural role of Be can be compared with that of Al in the aluminosilicates. We report here on the preparation and X-ray crystal structure determination of  $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$ , which contains planar  $\text{BeO}_3$  groups. Such coordination is unusual for beryllium, and, in complex oxides, has apparently been found previously only in  $\text{Y}_2\text{BeO}_4$  (ref. 2),  $\text{Ca}_{12}\text{Be}_{17}\text{O}_{29}$  (ref. 3), and  $\text{K}_2\text{BeO}_2$  (ref. 4).

The compound  $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$  was prepared by solid-state reaction between appropriate quantities of  $\text{Rb}_2\text{CO}_3$ , BeO and quartz. A single crystal was selected from a sample which had been heated at 950 °C for three days. The orthorhombic cell dimensions are

$$a = 8.92 \text{ \AA}; b = 8.32 \text{ \AA}; c = 5.15 \text{ \AA}$$

The intensity data, obtained from a Hilger and Watts Y-190 linear diffractometer with  $\text{MoK}\alpha$  radiation, indicated a body-centred space group. The solution of the vector (Patterson) map indicated the space group to be  $\text{I}2\text{mm}$ . Later, a few, rather weak, additional reflections were observed on films, and the space group  $\text{P}2_1\text{nn}$  was adopted. The complete solution of the structure using vector and Fourier methods, followed by least squares refinement, has so far given values of  $R = 0.071$ , over 241 independent reflections.

The structure consists of  $\text{Si}_2\text{O}_7$  groups, linked at their corners by Be, to form an infinite framework anion of empirical formula  $[\text{Be}_2\text{Si}_2\text{O}_7]^{2-}$ . Large cavities contain two crystallographically distinct  $\text{Rb}^+$  ions, which have 13 and 14 nearest neighbour oxygen atoms (within 3.8 Å), respectively. In the planar  $\text{BeO}_3$  group, Be–O distances are around 1.54 Å, which is about 0.11 Å shorter than those in tetrahedral  $\text{BeO}_4$  (ref. 5); O–Be–O angles are close to 120°.

X-ray powder diffraction data suggest that  $\text{Cs}_2\text{Be}_2\text{Si}_2\text{O}_7$  is isostructural with  $\text{Rb}_2\text{Be}_2\text{Si}_2\text{O}_7$ .

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## Coccolith blooms in the Kimmeridge Clay and origin of North Sea Oil

IN this paper I suggest that Kimmeridge Clay oil shales (and therefore much of the oil in the North Sea) were formed from algal blooms, in an environment between open ocean, and an enclosed marine basin. These conditions favour the production of such blooms, which by deoxygenating and poisoning the water, could temporarily create bottom conditions suitable for the formation of organic-rich sediment.

Thin coccolith-rich bands were first noted in the Kimmeridge Clay by Downie<sup>1</sup>, at four levels in the cliff sections near Kimmeridge Bay, Dorset. The most prominent of these bands, the White Stone Band, was subsequently recorded in boreholes in Hampshire, Surrey, Sussex<sup>2</sup> and Norfolk<sup>3</sup>.

Coccolith-rich bands of a similar nature have recently been recorded at three levels in a borehole at Donington on Bain, Lincolnshire, each level corresponding with one of the higher levels in Dorset. Re-examination of borehole material from Surrey and Norfolk has shown that the same three horizons are present in Surrey, and that the lowest two of these are present in Norfolk (Fig. 1). Thus, these coccolith-rich bands occur over an area where the Kimmeridge Clay varies in thickness from < 100 m to > 500 m, and which embraced both the stable margins and the more rapidly subsiding parts of the basin of deposition.

The coccolith-rich bands are readily recognisable in borehole cores by their pale colour and lightness in weight, and at outcrop by their almost white weathering patina. In Dorset the thickest band is ~ 0.5 m thick, but elsewhere the three bands are each generally < 0.1 m thick. The coccolith contents of these bands ranges from 60–98% of the calcium carbonate fraction of the rock (30–> 50% of the whole rock). Most other Kimmeridge Clay lithologies contain < 3% coccoliths/whole rock, although some of the more calcareous clays have yielded up to 12.5% coccoliths/whole rock.

The coccolith-rich bands are generally composed almost wholly of one species, *Ellipsagelosphaera britannica* (Stradner)<sup>3</sup>. Even now, seasonal blooms of coccoliths consisting largely of one species occur from time to time over large areas of the North Sea<sup>4</sup>. Such blooms characteristically form in seas which are rich in land-derived nutrients and are to some degree land-locked so that, although they remain a fully marine environment, their salinities are slightly lower than those of the open oceans. The persistence and composition of the thin coccolith-rich beds in the Kimmeridge Clay suggests that they were formed from similar blooms.

In addition to their usefulness as stratigraphical marker beds the coccolith-rich bands may provide an insight into the conditions of depositional environment in which the Kimmeridge Clay oil shales were deposited, since each of the coccolith-rich bands recorded to date occurs in close association (usually interlaminated) with oil shales.

Although they vary considerably in thickness, the Kimmeridge Clay sequences in Dorset, Surrey and Norfolk can be matched with one another in lithological and faunal detail<sup>3</sup>, suggesting that ecologically similar environments existed over much of southern England, in spite of great variations in rate of subsidence of the sea floor. The distribution of the Kimmeridge Clay lithologies and fauna, mostly pelagic ammonites and benthonic bivalves, including oysters, preserved in calcite, suggests accumulation in a relatively shallow marine environment on a broad continental shelf. The oil shales contain a richer and more abundant fauna than any other lithology in the Kimmeridge Clay. They are characterised by bedding planes crowded with one or two species of small bivalve or ammonite preserved in either calcite or pyrite, and by small gastropods, phosphatised fish debris and faecal pellets, abundant Foraminifera and, locally, by pyritised plates of the free-swimming crinoid *Saccocoma*.

In Dorset the oil shales contain ≤ 40% of brown organic matter which Forsman<sup>5</sup> has described as composed mostly of kerogen. This kerogen occurs largely as laths of amorphous organic matter (AOM) but is rich in palynomorphs such as dinoflagellates<sup>1</sup>. Cosgrove<sup>6</sup> recorded abnormally high iodine and bromine contents in some of the oil shales from the Kimmeridge Clay in Dorset, and has related this to the abundance of microplankton present.

According to Trask<sup>7</sup>, few modern typically marine sediments contain more than 10% of organic matter; near-shore sediments generally contain between 1% and 8% and open-ocean sediments ~ 1%. Kaplan and Rittenberg<sup>8</sup> state that the

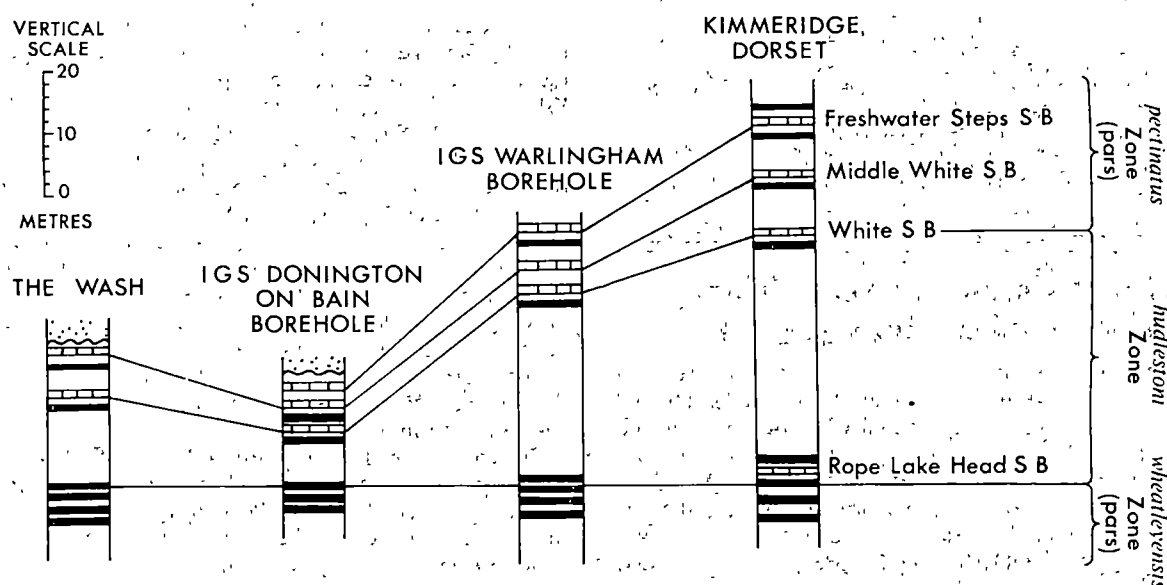


Fig. 1 Correlation of coccolith-rich bands referred to in the text

highest organic content recorded now in any marine environment is 23.4% in one of the Norwegian fjords. The oil shale fauna, although limited in variety, is fully marine, and clearly does not represent an anaerobic bottom environment comparable to that of any modern closed or restricted basin.

The apparent paradox of substantial amounts of organic material being preserved in sediments which, on general faunal and sedimentary grounds, seem to have been laid down in a shallow, marine environment was first recognised by Hallam<sup>9</sup> in the Lias. The solution he suggested was that of anaerobic bottom conditions developed beneath a layer of undisturbed, effectively stagnating, seawater<sup>9,10</sup>.

There are a number of difficulties in applying this suggestion to the oil shales in the Kimmeridge Clay. First, there is evidence of a plentiful benthonic fauna in the oil shales, both in the form of burrowfill structures and bivalve and gastropod shells. Second, the oil shales are thin seams (generally < 0.1 m-thick) which occur over a wide area (> 200,000 km<sup>2</sup>), apparently independent of distance from land and the overall shape of the sea floor. Thirdly, the oil shales occur in juxtaposition (commonly interburrowed) with a variety of lithologies which must represent a variety of differing bottom conditions.

The stagnant bottom conditions hypothesis could explain very local occurrences of oil shale within a single basin of

deposition containing variable bottom conditions, or could explain widespread occurrences within a basin containing uniform conditions. It does not, however, explain the widespread occurrences in the Kimmeridge Clay, which embrace several depositional basins containing variable conditions. Nor does it explain the characteristic association of these oil shales with the occurrences, in flood proportions, of the pelagic flora and fauna of coccoliths, Foraminiferida, ammonites and the crinoid *Saccocoma*.

Dinoflagellate blooms have been recorded from a number of present-day localities, mostly on continental shelves, where their relationship to modern AOM-rich sediments has been studied. The abundance and types of palynomorph present in any given area are governed by complex and interacting factors, such as water turbulence, distance from shore, availability and proximity of river effluence and upwelling<sup>11</sup>. Such blooms are generally dominated by one or two species and give rise to AOM-rich sediments. The ability of certain types of dinoflagellate bloom to poison the benthonic fauna of an area is well known<sup>12</sup>, and it seems likely that if such poisoning took place on a sufficiently large scale, anaerobic bottom conditions, conducive to the formation of pyrite and phosphate, would be temporarily created.

Thus, by analogy with the origin of the coccolith-rich beds,

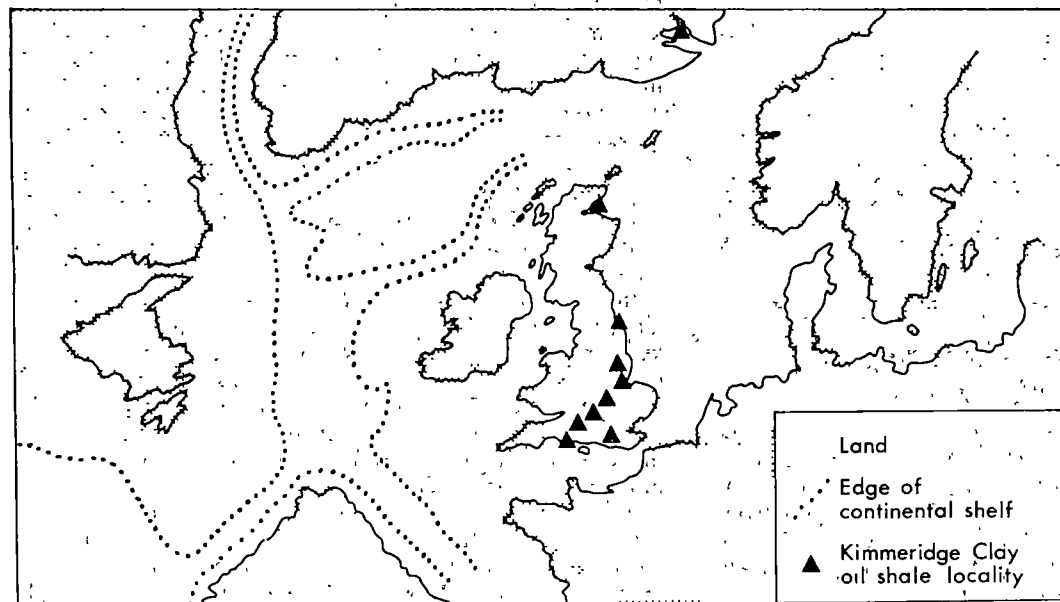


Fig. 2 Palaeogeography in middle Kimmeridge Clay times: continent fit after Laughton<sup>14</sup>.

with which they are so intimately associated, the Kimmeridge Clay oil shales appear to have resulted from algal (probably dinoflagellate) blooms. The combined faunal and sedimentary evidence suggests that the organic content of the shales is largely derived from blooms, and that the blooms themselves, by deoxygenating and poisoning the water, provided the temporary anaerobic bottom conditions required to ensure the preservation of their organic content. It is perhaps significant that one of the bivalves most affected by the 1968 toxic bloom off the east coast of Britain was *Lucinoma borealis* Linne<sup>12</sup>, and that the bivalve which forms the most extensive plaques in the Kimmeridge Clay oil shales is '*Lucina*' *minuscule* Blake.

Thick oil shale seams occur only in the upper part of the Lower Kimmeridge Clay and the lower half of the Upper Kimmeridge Clay. At these levels the fauna is less diverse than that at lower levels in the Kimmeridge Clay, and seems to represent a less open marine environment. The highest Kimmeridge Clay, above the Rotunda Nodule Bed, contains little bituminous matter and was probably deposited in an even more restricted basin which formed as a result of the Middle Volgian earth movements<sup>13</sup>.

The Kimmeridge Clay oil shales appear therefore to have been deposited in an environment intermediate between open ocean and restricted marine basin at a time when the delicate balance between palaeogeographical influences and nutrient supply was particularly favourable to the formation of algal blooms. The palaeogeography in Kimmeridge Clay times was probably not unlike that of the present-day North Sea, with a series of subsiding basins linked by relatively narrow straits between land areas of low relief (Fig. 2). In the Mesozoic rocks of the British land area, similar palaeogeographical conditions seem to have existed only in the Lias and the Oxford Clay, and the bituminous bands in those formations may have a comparable origin to those of the Kimmeridge Clay.

The likelihood of the Kimmeridge Clay being a major oil source rock off the western coast of Britain would seem to be largely dependent on the nature of the Kimmeridgian palaeogeography. If this area had access to a fully oceanic environment of a proto-Atlantic sea (Fig. 2), then the prospect of thick oil shale seams and a major oil source occurring at this stratigraphical level is unlikely to be high.

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## Dichotic verbal transformations and evidence of separate processors for identical stimuli

AFTER listening for several seconds to a clear recording of any word or phrase repeated over and over, a listener experiences illusory changes called "verbal transformations" (VTs), and hears a series of abrupt transitions, sometimes to new forms, sometimes back to the actual stimulus or to

illusory forms reported earlier<sup>1</sup>. Considerable phonetic distortion may take place, so that with the repeated stimulus 'tress' listeners can believe they are hearing clearly words as far removed as 'purse' or 'florist'. The temporary receptive aphasia and perceptual reorganisation produced by VTs have provided access to mechanisms for speech processing not otherwise available for study<sup>2</sup>. Previous investigations have been restricted to one repeated statement at a time; we have extended this research with VTs to investigate dichotic presentation of separate stimuli to each ear.

Our initial observations of dichotic VTs involved presentation of a different repeated word to each ear. We found that subjects had no trouble monitoring both ears, and that VTs seemed to occur in an uncorrelated fashion on both sides. A more interesting condition was suggested by this observation. What if the dichotic stimulus consisted of the same word delivered asynchronously to each ear? By separating the presentation of the single repeated word to each ear by exactly half the duration of the word (a few hundred milliseconds), fusion into a single auditory image would be prevented, and identical stimuli with temporal cross-ear symmetry would be received by each ear. Would VTs occur independently for each side? The answer would have some theoretical interest.

If identical reorganisation to another word occurred at the same time in each ear, it would indicate that a single processor responsible for verbal identification was being used for the input to the two ears. Convergence of dichotic verbal input on the same speech processor has been suggested by some investigators working with the "right ear advantage" effect for speech perception. In studies demonstrating this ear advantage, different verbal messages delivered simultaneously to each ear were shown to be handled asymmetrically, with input to the right ear identified somewhat more accurately<sup>3,4</sup>. In explanation, it has been suggested that there is competition for access to a single speech processor (located in the left cerebral hemisphere for most right-handed subjects), and that the pathway from the contralateral right ear is favoured over the pathway from the ipsilateral ear<sup>5,6</sup>.

Alternatively, if VTs with the temporally dichotic but acoustically identical stimuli occurred independently (so that specific repetition-induced phonetic distortions were unilateral), it would suggest that functionally separate verbal decoders or processors were handling the identical input received by each ear. This outcome would raise the possibility that the results of the earlier studies on the right ear speech advantage do not reflect favoured competition for engaging a single processor, but might rather follow from different characteristics of functionally distinct verbal processors, each handling one of the simultaneous messages.

The experimental stimuli were prepared from a single statement of the word 'tress' repeated each 492 ms by a loop of recorded tape. A dual output digital delay line was used to split the repeated signal into two identical statements separated by half the word duration (246 ms), and a two-track recording was made with this fixed asynchrony. When played back through matched stereophonic headphones, the identical statements on the two sides were temporally symmetrical, including the contralateral accompaniment for each part of a lateral statement.

Separate groups of college students were used for each experimental condition shown in Table 1, and all (diotic control and three dichotic groups) heard the word 'tress' repeated for 5 min. The 20 subjects in the diotic group listened to the repeated word from one of the recorded channels delivered synchronously to each ear. These diotic subjects were unselected for handedness, but because of the correlation between handedness and the side of the dominant speech hemisphere<sup>7</sup>, the 100 subjects used for dichotic listening were all right-handed with no left-handed



Table 1 Verbal transformations with temporally dichotic presentation of a single stimulus

	n	Transitions mean (s.e.)	Forms mean (s.e.)	Proportion of simple changes*
'Tress' (diotic)	20	39.5 (4.90)	6.00 (0.62)	0.418 ± 0.035
'Tress' (dichotic, report one)†				
Right only	40	26.1 (3.38)	5.58 (0.49)	0.289 ± 0.028
Left only	40	23.0 (2.73)	5.50 (0.57)	0.371 ± 0.032
'Tress' (dichotic, report both)†				
Right response	20	14.7 (4.31)	4.15 (0.44)	0.186 ± 0.046
Left response	20	7.1 (1.25)	3.30 (0.26)	0.369 ± 0.086
Total (right + left)	20	21.8 (5.06)	4.85 (0.36)	0.242 ± 0.043

\*The range given for proportion of simple changes corresponds to the 95% confidence intervals.

†Delivery to each ear separated by half the duration of the repeated word.

siblings or parents. The instructions were similar to those used in earlier studies<sup>7</sup>. Subjects called out what the voice said, and any changes heard; the experimenter wrote down all responses, later checking the transcription against a tape recording. Forty dichotic subjects were instructed to report only words heard on the right side ('report right'); another 40 were instructed to report only words on the left ('report left'). The final (and most interesting) group consisted of 20 subjects who were instructed to monitor both ears ('report both') and to report all changes, using a hand-operated indicator to show the side on which each reported change occurred. This group did not find it difficult to monitor both ears.

The major finding was that VTs occurred independently on each side. Each of the 20 subjects listening dichotically and monitoring the identical stimuli on both sides reported hearing phonetically different words on the two sides at the same time for some period during the test. As an example, a listener reported hearing the stimulus change from 'commence' back to 'tress' in one ear while the other continued to hear 'tress' repeated over and over. Such independent changes demonstrate that separate speech processors operating up to the level of syllables or words were handling the identical input to each side.

By examining the characteristics of verbal reorganisations under the various experimental conditions, we can compare properties of these processors. In keeping with earlier studies, the results summarised in Table 1 are scored in terms of transitions (perceptual changes) and forms (different perceptual organisations). Individual scores for these two measures are not correlated: as long as an individual reports at least two forms he can achieve a high transition score by rapid changes from one form to the other. After logarithmic transformation<sup>7</sup>, analysis of variance showed that transition scores were significantly higher for diotic listening than for report left, and also significantly greater than left and right combined for report both ( $P < 0.05$ ). There was no appreciable difference between report right and report left only, and for report both, the difference between the right response and the left response, although sizeable, was not significant. The decrease in numbers of illusory changes observed for dichotic relative to diotic listening may involve attentional factors related to the greater complexity and difficulty of the dichotic task. An earlier study<sup>1</sup> indicated that making the task more difficult by the addition of masking noise to a diotic signal also decreased the rate of VTs.

An analysis of variance for numbers of forms showed no significant differences between any of the groups. But if we consider the nature of the changes made by individuals when going from one form to the next, an interesting difference is found. When a listener hears the stimulus word 'tress' change to 'dress' or the illusory word 'frazzle' change to 'trazzle', we have examples of a simple change with one phoneme transformed in each case. Complex changes involve two or more phonemes, and may be as

extreme as the transition reported from 'stress' to 'conniving' in which all phonemes change. The proportion of simple changes for group totals in each experimental condition is shown in Table 1, together with the 95% confidence limits for each proportion. The proportion of simple changes for diotic stimulation was significantly greater than right and left combined for the dichotic report both condition. Within the dichotic report both group, the proportion of simple changes was significantly greater for left than for right reports, as if the left side were more conservative.

Using the asynchronous identical stimuli, it was necessary to introduce each to separate ears to obtain independent VTs. If the outputs of the two stimulus channels were mixed and then introduced dichotically to both ears, the mixed signal was not intelligible. With continued listening, the mixed input became organised into a clearly heard single word which then underwent illusory changes characteristic of repeated single words.

Little is known concerning the nature of neural decoding of speech signals; our experiments with dichotic verbal transformations do not inform us of the spatial relation of the separate processors, but merely of their presence and characteristics. It seems that, since more than one processor can be used for identical auditory inputs, we need not consider that the right ear advantage, reported by many investigators for dichotic messages, reflects competition for a single processor as is commonly believed. It seems rather that, if one processor is occupied with a particular task, others may be assembled for similar analyses. The number of potential verbal processors may not be limited to two: in a preliminary experiment with James Bashford we delivered three asynchronous versions of one repeated word through headphones. Each version was preceded and followed by other versions separated by one-third the word duration, with one sequence of the repeated word heard on the left, one on the right and one in the medial plane (the centre signal was delivered dichotically; the lateral signals corresponded to separate monaural delays). Monitoring all positions was more difficult with three than with two statements, but all five experienced listeners tested heard the three versions each change independently.

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## Correspondence between sharp tuning and two-tone inhibition in primary auditory neurones

THE sharp frequency selectivity of single primary auditory neurones in mammals contrasts with the poor mechanical tuning of the basilar membrane<sup>1,2</sup>. This discrepancy has generally been explained by postulating some filter mechanism interposed between the basilar membrane and the excitation of auditory afferents<sup>3,4</sup>. A further property of these neurones which probably cannot be explained by basilar membrane behaviour alone is two-tone inhibition whereby the simultaneous presentation of a second tone reduces the response of a neurone to a first tone at the best, or characteristic frequency<sup>5-7</sup>. It has been suggested that this inhibition may also be a property of the additional cochlear mechanisms which follow the basilar membrane<sup>8</sup>. I now present evidence that the loss of sharp frequency selectivity of primary neurones is intimately linked to changes in two-tone inhibition and that the two phenomena might be related to a common mechanism which is vulnerable to perilymph removal.

In 15 guinea pigs anaesthetised with Nembutal, data were collected from 57 single primary neurones in the spiral ganglion of the cochlea<sup>9</sup>. The measure of frequency selectivity estimated from the conventional tuning curves was the  $Q_{10\text{ dB}}$  value (the characteristic frequency divided by the bandwidth of the tuning curve 10 dB above threshold at the characteristic frequency). Two-tone inhibition was studied in the same neurones by computing time histograms of the number of action potentials in response to tone bursts. The first tone at the characteristic frequency was presented for 50 ms while the second, inhibiting tone, started 10 ms after the first and lasted for 30 ms (see Fig. 1). Both tones had rise-fall times of 5 ms. The inhibitory regions on either side of the characteristic frequency were mapped out as completely as possible in the time available for each unit. Inhibition was designated "strong", "weak" or "absent" when the second tone, when maximally effective, could reduce the firing rate to the first tone by either more than 50%, by less than 50%, or not detectably, respectively. The first tone was fixed at an intensity of 10-15 dB above the characteristic frequency threshold.

For 33 neurones, the inhibitory regions were thoroughly studied and the strength of inhibition determined by the above criteria and compared with variations in the sharpness of the single-tone tuning curves found from one animal to another<sup>9,10</sup>. These data, shown in Fig. 1, reveal a close association between the sharpness of neural tuning and the strength of two-tone inhibition.

Furthermore, the removal of perilymph from the scala tympani caused a reversible loss of sensitivity of about 20 dB and a broadening of the tuning curve in cells held throughout the procedure<sup>11</sup>. In all the cells observed, this effect was accompanied by the disappearance of two-tone inhibition (Fig. 2). Complete data were obtained from four cells in three animals with partial confirmation from other cells. Allowing the perilymph to refill the scala tympani caused a rapid return of sharp tuning and two-tone inhibition. Intensities of characteristic frequency tone less than 10 dB above threshold were tried in the drained cochlea, to see if inhibition was perhaps still present but weak. No inhibition could be demonstrated. A complete range of intensities of the second tone were also used, also

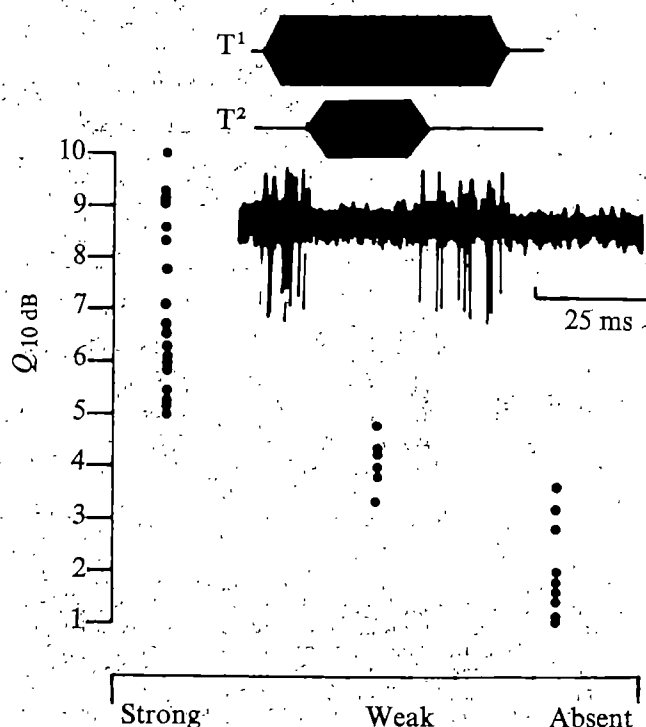


Fig. 1. Tracing shows an example of strong two-tone inhibition in a single spiral ganglion. The approximate positions of the two-tone bursts are shown above the tracing. Three sweeps of the oscilloscope are superimposed. The graph shows the relationship between the sharpness of tuning curves (the  $Q_{10\text{ dB}}$ ) and the strength of two-tone inhibition (see text) for 33 cells in 15 animals.

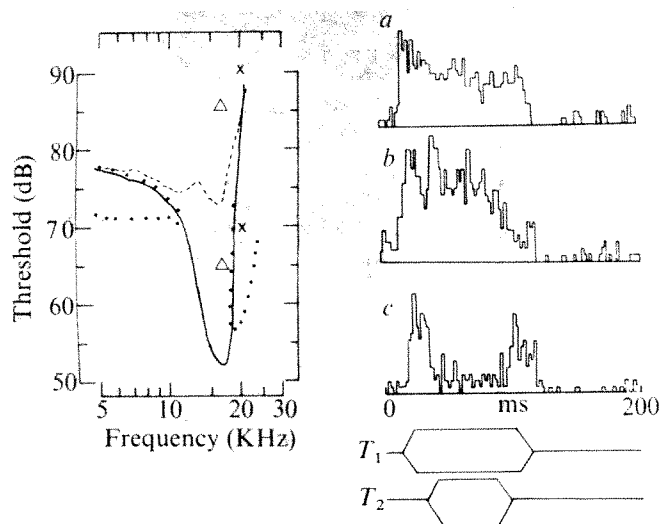
without demonstrating any appreciable inhibition. Thus two-tone inhibition was totally absent on both the high and low-frequency sides of the abnormally drained tuning curves.

One explanation that should be considered for these results is that the inhibition is lost because the lower slopes of the broadened tuning curves cause an encroachment of the excitatory areas into the inhibitory regions. This is certainly a possibility for the portion of the low frequency slope where the slope changes dramatically during drainage.

For the high frequency slopes, part of the inhibitory regions shown in the tuning curve of Fig. 2 would still fall outside the abnormally broad tuning curve, even if they too were elevated by 20 dB by perilymph drainage. All combinations of characteristic frequency and second tone failed to elicit inhibition. Figure 2b illustrates the only effect that can be produced by the second tone in the drained cochlea. This is an excitatory effect superimposed on the response to the characteristic frequency tone as the intensity of the second tone crosses the boundary of the abnormally broad tuning curve.

Though a more detailed mapping of inhibitory regions than was possible in the present study may reveal some encroachment of the broad tuning curve on to inhibitory regions on the high frequency slope, I believe the present results are sufficient evidence for a genuine loss of two-tone inhibition associated with loss of sharp frequency selectivity.

Thus, loss of sensitivity, loss of inhibition and loss of sharp frequency selectivity seem to go hand in hand. These parallel variations might be explained by alterations in the functioning of a common mechanism. The dramatic and reversible effects of perilymph removal on both frequency selectivity and two-tone inhibition throw some light on the cochlear mechanisms which follow the broad tuning of the basilar membrane. It has been known for some time that the frequency selectivity of primary auditory neurones cannot be related to neurochemical synaptic interactions between



**Fig. 2** Graph on left shows the effect of perilymph removal on the tuning curve of a single ganglion cell. Solid line is the tuning curve before drainage, broken line is the curve with perilymph removed. The dotted lines demarcate the inhibitory regions for the cell in the normal case. *a*, *b* and *c* are histograms of the response of the same cell to 20 presentations of characteristic frequency ( $T_1$ ) and inhibitory tone ( $T_2$ ). *a*, The initial response to  $T_1$  alone at 17 kHz, 67 dB. *b*, Response with perilymph removed to  $T_1$  now 85 dB and  $T_2$  at 20 kHz, 90 dB. The excitatory effect seen during  $T_2$  presentation is the only form of interaction seen. *c*, Response to the same two frequencies with perilymph replaced.  $T_1$  now 65 dB,  $T_2$  70 dB. Strong inhibition is seen. Sound pressure is measured at the tympanic membrane (dB referred to  $0.0002 \text{ dyne cm}^{-2}$ ).

afferents<sup>12</sup>. One has therefore to consider physical mechanisms which could restrict the extent of excitatory input to these neurones arrayed along the cochlear turns. It was originally suggested that the action of perilymph removal might be to alter the mechanical response of the basilar membrane to acoustic stimulation<sup>11</sup>. Several authors have argued against this<sup>1,13</sup> and support the alternative explanation; that the underlying mechanism is an increase in the resistance of the scala tympani pathway. Such a resistance change would be expected to interfere with the spread of receptor currents between neighbouring regions of the basilar membrane. Whatever the real explanation turns out to be, the present evidence suggests that both sharp tuning and two-tone inhibition arise from the functioning of such a common mechanism.

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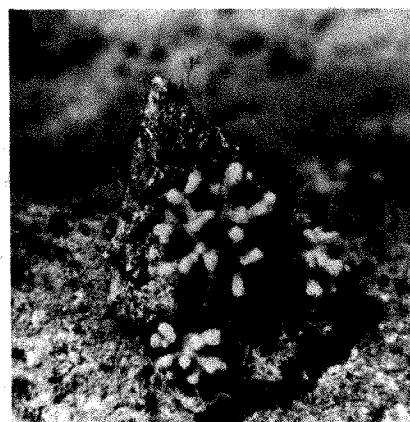
## The Red Sea coral *Stylophora pistillata* is an *r* strategist

MACARTHUR and Wilson<sup>1</sup> coined the terms *r* selection and *K* selection to describe two general kinds of selection they believed could be functioning in nature (*K* refers to carrying capacity and *r* to maximal intrinsic rate of natural increase,  $r_m$ ). As originally defined, the *r* and *K* selection concepts postulated that alternative genotypes within a species possessed somewhat divergent life history characteristics: genotypes with a high  $r_m$  were suggested to have a relatively low *K* and vice versa<sup>1</sup>. Thus, *r* selection occurs in a fluctuating environment when there is no crowding and it is more important to increase the size of the population. *K* selection occurs in stable environments when population size is always near the maximum and increasing efficiency in resource use is required for the production of a few but extremely fit offspring. Pianka<sup>2</sup> extended these concepts to include comparisons of individuals of different species. He postulated that no organism is completely *r* selected or completely *K* selected, but all must reach some compromise between the two extremes. Thus, a given species could be visualised along an *r*–*K* continuum and identified by a set of *r* and *K* characteristics<sup>2</sup>.

*Stylophora pistillata* (Esper), an important scleractinian coral in the Gulf of Eilat<sup>3</sup> has most of the *r* characteristics summarised by Pianka<sup>2</sup>—success in colonising unpredictable reef habitats, rapid development, great population turnover, early reproduction, high  $r_m$ , small body size, short life span, density independent mortality (often catastrophic), wide dispersal gradient and poor competitive ability. Most of the other scleractinian corals of Eilat have the opposite characteristics (*K* strategists), or are ranked below *S. pistillata* in their proximity to the *r* end of the *r*–*K* continuum.

The coral reefs of Eilat constitute a physically controlled coral community near the reef flat (the *r* end of the *r*–*K* continuum), existing side by side with the subtidal biologically accommodated community, increasing in diversity (and environmental predictability) to a depth of 50 m (ref. 3). The relative unpredictability of the reef flat at Eilat (in the sense of Slobodkin and Sanders<sup>4</sup> and Colwell<sup>5</sup>), compared with the deep reef, may be best demonstrated by the catastrophic and extremely low tides which occur periodically but unpredictably along the northern Gulf of Eilat. Thus, in September 1970, an unexpected and extremely low tide caused 80–90% mortality of the hermatypic coral communities on the reef flats of Eilat<sup>6</sup>. In spite of its small colony size<sup>3</sup>, *S. pistillata* is among the most important

**Fig. 1** *S. pistillata* colonies growing on the shell of the gastropod *Trochus dentatus*.



**Table 1** Effect of initial size of colony on growth rate

Size interval of radius ( $\bar{r}$ )*	Number of colonies measured	$\bar{d}_1$ (cm)†	$\bar{d}_2$ (cm)‡	$\frac{\Delta \bar{d}}{\text{yr}}$ (cm)§	$\frac{\Delta \bar{d}}{\text{yr}}$ %
0.01–0.50	20	0.840 (0.135)	5.104 (0.792)	1.909 (0.254)	227.26
0.51–1.00	52	1.543 (0.267)	7.170 (0.924)	2.520 (0.253)	163.32
1.01–1.50	32	2.536 (0.304)	7.800 (1.168)	2.357 (0.345)	92.94
1.51–2.00	30	3.546 (0.296)	8.958 (0.426)	2.423 (0.271)	68.33
2.01–3.00	32	4.978 (0.530)	10.682 (1.072)	2.554 (0.347)	51.30
3.01–4.00	9	6.892 (0.636)	12.510 (0.650)	2.516 (0.337)	36.50
4.01–5.00	9	8.714 (0.330)	14.055 (1.406)	2.392 (0.276)	27.45

s.d. given in parentheses.

\*Geometric mean radius  $\bar{r} = (l \times w \times h)^{1/3}/2$ .

†Geometric mean diameter at the beginning of measurements.

‡Geometric mean diameter at the end of measurements.

§Mean increase in diameter per year =  $365(\bar{d}_2 - \bar{d}_1)/815$ .

||Mean % increase in diameter per year =  $\left(\frac{\Delta \bar{d}}{\text{yr}} / \bar{d}_1\right) \times 100$ .

frame builders of the reef flat because of its abundance<sup>7</sup>. Before the catastrophic low tide of 1970, it composed ~25% of the total living coverage of the reef flat of the nature reserve at Eilat. During the low tide, 98% of *S. pistillata* colonies on the reef flat were killed, while other abundant species were somewhat less affected<sup>8</sup>.

In such uncertain conditions, the optimal strategy is to put all possible matter and energy into reproduction, and to produce as many progeny as possible<sup>8</sup>. Histological examination of gonads, in collections made every 2 weeks for a year, revealed that *S. pistillata* breeds during the 8 months from December to July. Colonies 4.0–5.0 cm in diameter already contained ripe eggs<sup>9</sup>. Three years of periodical observations of larval settlement, development and growth indicated that colonies of this size are between 2 and 3 yr old (unpublished). The only other coral species for which estimates have been made of the time required to reach sexual maturity are *Favia doreyensis* (8 yr) and *Fungia actiniformis* (10 yr) in the Great Barrier Reef<sup>10</sup>. In general, reduction of the age for first reproduction and an increase in progeny number tend to increase  $r$  (ref. 11). Envelopment of *S. pistillata* colonies with plankton nets facilitated the capture of the released planulae, and showed

that a moderate-sized colony may produce 100–400 planulae within 2 h after sunset<sup>9</sup>.

Perhaps the most important feature of *S. pistillata* as an  $r$  strategist is that it is usually the pioneer coral species colonising 'new environments' or unexploited habitats. Such species have variously been called 'fugitive'<sup>12</sup>, 'opportunistic'<sup>13</sup>, 'colonising'<sup>14</sup>, 'weedy'<sup>15</sup>, or ' $r$  selected'. The seawater system of the Marine Biological Laboratory at Eilat provided a good opportunity to examine the fast-colonising capabilities of this species. Ninety-five colonies of *S. pistillata* were counted in 1972 on the seawater pipe (installed in 1967, 20 m long, 30 cm in diameter), and about 100 colonies on the concrete blocks used to hold down the pipe. Other hermatypic species counted on the seawater system were *Millepora dichotoma* (ten colonies), *Favia fava* (five colonies), *Cyphastrea microphthalma* (two colonies) and *Acropora variabilis* (one colony). A new seawater pipe, installed parallel to the old pipe was colonised by 45 colonies of *S. pistillata* 8 months later. In April 1975, 92 colonies of this species were recorded, and in August 1975, 173 colonies of this species were counted on the same pipe. So far, no other scleractinian corals have settled on the new pipe. Another demonstration of the opportunistic colonising character of *S. pistillata* is the fact that it may be found growing in shallow lagoons attached to the roots of the mangrove tree *Avicennia maritima*, where water temperature during low tide reaches 36 °C and salinity 48‰. It may also be found attached to the shells of mobile animals, such as the gastropod *Trochus dentatus* (Fig. 1) or to any other newly introduced foreign matter on the reef.

The growth rate of *S. pistillata* is among the fastest of the scleractinian corals in the Gulf of Eilat (Y.L., unpublished and L. Fishelson, personal communication). The growth rate of 134 colonies, of various sizes, of *S. pistillata* was studied during 815 d (May 17, 1973–August 10, 1975). Each colony was numbered by a plastic tag and length, width and height were measured approximately every 3 months. Length is chosen as the distance across a coral between the tips of the branches which are furthest apart. Width is chosen as the distance perpendicular to the length axis. Height is chosen as a measure perpendicular to the length and width axes. When colonies died during the study, 1 tagged and measured new and healthy colonies of similar dimensions growing nearby. The shape of *S. pistillata* approximates a sphere. The colonies were, therefore, divided into size groups according to the geometric mean of their radius ( $\bar{r}$ ). Although the range of the mean increase in diameter per year ( $\Delta \bar{d} \text{ yr}^{-1}$ ) was very narrow (1.9–2.5 cm), among the size groups studied (Table 1), the

**Fig. 2** Healthy colonies of *S. pistillata* (branched colony) and *F. fava* (massive colony) were brought into physical contact on October 7, 1974 (left). On January 1, 1975, the terminal tissues of the branches of *S. pistillata* which touched the *Favia* colony were seen to be killed (right). *S. pistillata* branches within a distance of 2.0–2.5 cm from the *Favia* colony were also killed, which might indicate the furthest distance the mesenterial filaments of *F. fava* can reach. Note that the differences in magnification of the two photographs are due to the use of different lenses of the Nikonos II underwater camera. The left photograph was taken with a 35-mm lens, while the right photograph was taken with a 28-mm lens.





smallest colonies increased in absolute size significantly less compared with the larger ones ( $t$  tests,  $P < 0.05$ ). The rate of growth per year of the largest size category, however, was approximately eight times slower than that of the smallest category, which might indicate that like all other organisms they grow proportionately more slowly as they age. The largest colonies of *S. pistillata* do not reach a geometric mean diameter greater than 30–35 cm (ref. 3). It is possible that when a colony reaches the size of sexual maturity (diameter of 4.0–5.0 cm) much of its energy is channelled into the production of sexual cells rather than growth, which considerably lowers the growth rate. Regeneration in this species is also very fast: some accidentally broken branches grew 5.0–7.0 cm yr<sup>-1</sup>. During this study, 80–85% of the original population of *S. pistillata* died. The causes of mortality were mainly abiotic (winter storms or heavy sedimentation). Nevertheless, many new colonies settled in the same study area. Thus, *S. pistillata* has a short life span and high population turnover in comparison to the other coral species in the same area.

Although the vast majority of the hermatypic corals at Eilat are limited to the deep reef (20–50 m depth) and are rare, *S. pistillata* is very abundant and shows a wide range of dispersal, from the lagoon to the deepest point of the reef. When space becomes limiting, however, *S. pistillata* is the first to be competitively excluded by the deep reef coral specialists.

The area exposed to light is one of the most important limiting factors on a coral reef. Corals compete with each other for available space in various ways<sup>10</sup> one of which takes the form of aggressive behaviour<sup>10</sup>. Observations and experiments (Fig. 2) with *S. pistillata* rank this species among the lowest in aggressive hierarchy. That is, on physical contact with most of the other hermatypic corals it is competitively excluded. Abundant species which occur together with *S. pistillata* on the reef flat proved to be more aggressive. Thus, species such as *F. favus*, *Platygyra lamellina*, *Echinopora gemmacea*, *Goniastrea retiformis*, *Pocillopora danae*, *M. dichotoma*, and many others can be seen to extrude their mesenterial filaments and digest away the living tissue of the neighbouring *S. pistillata*. No damaging effects have been observed, however, when several colonies of *S. pistillata* were brought into physical contact with each other.

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## Cell differentiation by 3',5'-cyclic AMP in a lower plant

Cyclic AMP evokes or mediates a multitude of responses in animals and microorganisms<sup>1,2</sup>. Whereas the occurrence and regulatory role of cyclic AMP in these organisms is well established, little is known about its role in plants. Cyclic AMP has been reported to mimic certain effects of gibberellins, indoleacetic acid (IAA) and phytochrome in higher plants but in no case has its endogenous presence been unequivocally demonstrated<sup>3,4</sup>. We describe here a new response of cyclic AMP at the cellular level in the moss protonema; the results show that it is involved in the differentiation of chloronema cells. The intracellular level of cyclic AMP in these cells is four- to sevenfold higher as compared with that in caulonema cells. In a leaky chloronema-repressed mutant isolated by us, cyclic AMP is shown to enhance the differentiation of chloronema filaments.

The protonema constitutes the juvenile phase in the life cycle of a moss and has been widely used for studies on cell differentiation<sup>5</sup>. In the moss *Funaria hygrometrica*, two distinct cell types are formed during protonema development<sup>6,7</sup>. A spore germinates producing a multicellular filament. Each chloronema cell contains a large number of chloroplasts and has septa at right angles to the longer axis. Later, caulonema cells are formed; these have fewer chloroplasts and show oblique septa. The buds (initials of moss plants) differentiate only on caulonema cells and their formation is enhanced by cytokinins<sup>7</sup>. The protonema can also be regenerated from any part of a gametophore or a sporophyte, but caulonema cells always differentiate only after the chloronema cells have been formed<sup>8,9</sup>.

The experiments were carried out with the wild-type cell line J-2 of the moss *Funaria hygrometrica* (L.) Sibth grown in axenic cell suspension cultures<sup>10</sup>. In the absence of

Table 1 Effect of cyclic AMP and IAA on the differentiation of chloronema and caulonema filaments

IAA ( $\mu$ M)	% Chloronema filaments Cyclic AMP ( $\mu$ M)					
	0	5	10	50	100	500
0	94	97	97	96	96	98
0.5	62	73	80	86	97	—
1.0	33	52	73	68	91	93
2.0	33	45	52	61	—	—
5.0	21	30	39	49	—	—

Cells were cultured in minimal medium (MM) at  $26 \pm 2^\circ \text{C}$  in continuous light at an initial cell density of  $20 \text{ mg l}^{-1}$  as described previously<sup>10</sup>. Cyclic AMP sterilised by filtration through membrane filters was added to the autoclaved MM  $\pm$  IAA. After 6 d, at least 500 filaments were scored to determine the proportion of chloronema filaments.

exogenous auxins, protonema grown in liquid culture consist predominantly of chloronema filaments. Auxins enhance the differentiation of caulonema filaments<sup>11</sup>. In cultures grown in the presence of IAA, adenine derivatives other than cytokinins were observed to enhance the differentiation of chloronema filaments, thus antagonising the effect of IAA.

Formation of chloronema filaments was profusely enhanced by cyclic AMP and at a concentration of  $100 \mu\text{M}$ , their percentage was restored equal to that in control (Table 1). This increase was due to initiation of a large number of new chloronema branches and not due to a dedifferentiation of existing caulonema filaments. The latter continued to produce caulonema cells and this result strongly suggests that caulonema differentiation is independent of cyclic AMP.

**Table 2** Effect of various nucleotides and inhibitors of phosphodiesterase on the formation of chloronema and caulonema filaments in wild-type and mutant *pg-1* protonema

Compound	% Chloronema filaments	
	Wild type	<i>pg-1</i>
Nucleotides		
None	35	27
3',5'-cyclic AMP	91	66
Monobutyl cyclic AMP	91	37
Dibutyl cyclic AMP	78	45
Cyclic GMP	78	41
Cyclic IMP	59	35
Cyclic CMP	40	26
Adenosine	71	24
5'-AMP	59	24
5'-GMP	81	26
Phosphodiesterase inhibitors		
Theophylline	85	40
Aminophylline	95	42
ICI 58,301*	88	42

\*3-Acetamido-6-methyl-8-*n*-propyl-5-triazolo[4,3-*a*]pyrazine.

Mutant *pg-1* was isolated as follows. Spores ( $1.52 \times 10^5$  per 3 ml) suspended in MM+0.001% Tween-20 were exposed for 45 min to ultraviolet light from a Philips germicidal lamp (1.25% of the spores survived). After exposure, spore suspension was stored in dark (30 h) and then plated on complete medium. The protonema that grew 0.5 cm or more in diameter in 10 d (650) were screened for altered differentiation of various cell types. Three isolates behaved differently and only in one of these (designated *pg-1* here) caulonema filaments were found to be formed in the absence of IAA. The detailed method of isolating mutants will be published separately. Wild-type protonema was grown in MM+1  $\mu$ M IAA, whereas *pg-1* was cultured in MM+1% glucose. All nucleotides and inhibitors of phosphodiesterase were sterilised by filtration through membrane filters and then added to the autoclaved medium. A wide range of concentrations were tested; data are given only for 0.1 mM. Other details as in Table 1.

Mono- and dibutyl cyclic AMP, purine nucleotides and inhibitors of cyclic nucleotide phosphodiesterase also enhanced chloronema differentiation (Table 2). Theophylline and aminophylline, although active, inhibited the growth of cells at all the concentrations tested (0.01–1 mM). ICI 58,301 (an inhibitor of phosphodiesterase<sup>12</sup>) was not inhibitory and mimicked the effect of cyclic AMP. The pyrimidine derivatives were completely inactive. The compounds enhancing chloronema differentiation in the wild type may act by elevating endogenous levels of cyclic AMP, and an increase in the activity of adenylate cyclase by most of these has been shown in animal systems<sup>13,14</sup>.

Further evidence about the specific involvement of cyclic AMP in chloronema differentiation comes from the results obtained with the pale green mutant (*pg-1*), which we isolated by treating the wild-type spores (cell line J-2) with ultraviolet irradiation as described in Table 2. In suspension cultures, this mutant produced predominantly caulonema filaments (>70%) without the addition of an auxin (the

rest being the chloronema filaments). It may be recalled that in the wild type very few (<10%) or no caulonema filaments are formed in the absence of an auxin. In the mutant *pg-1*, cyclic AMP was quite active, whereas dibutyl cyclic AMP and phosphodiesterase inhibitors were marginally active (Table 2). Six days after addition of 0.1 or 1 mM cyclic AMP, a maximum of 66–70% chloronema branches were seen (control showed 27%). These branches arose as laterals on caulonema filaments and appeared similar in all respects to such branches from the wild-type protonema. Adenosine, 5'-AMP, 5'-GMP and other cyclic nucleotides did not alter the relative proportion of caulonema and chloronema filaments.

To elucidate the role of endogenous cyclic AMP, its intracellular levels were determined in the wild-type and the mutant protonema using the protein kinase assay<sup>15</sup> (Table 3). Detailed results concerning the characterisation of cyclic AMP in this moss will be published elsewhere. The endogenous concentration of cyclic AMP was four- to sevenfold higher in chloronema cells compared with a population of cells consisting of about 65–75% caulonema filaments. Cyclic AMP levels in the mutant and in the wild-type caulonema filaments were the same. A severalfold greater level of intracellular cyclic AMP in chloronema cells compared with that in caulonema filaments, and their enhanced differentiation by exogenous cyclic AMP, strongly suggest that cyclic AMP has a role in chloronema differentiation. The enhancement of chloronema filaments by adenosine and 5'-AMP in the wild-type protonema only (and not in the mutant) is a significant difference. Our results are consistent with the *pg-1* mutant being at least partially impaired in the synthesis of cyclic AMP.

Our results show that cyclic AMP enhances the differentiation of chloronema cells, whereas the auxins are already known to inhibit their growth and differentiation<sup>11,17,18</sup>. The chloronema cells contain IAA (M.M.J., unpublished), and a balance of endogenous cyclic AMP and auxin may in fact underlie the differentiation of chloronema cells. The results of the experiment in which the amount of cyclic AMP and auxin were altered in the medium support this suggestion; also, the percentage of chloronema filaments was greater at high cyclic AMP-IAA ratios.

The widespread occurrence of cyclic AMP among heterotrophic organisms<sup>4</sup> has led many investigators to test for its presence and function in plants<sup>3,4</sup>. So far, cyclic AMP or enzymes of its metabolism have been found in bacteria<sup>2</sup>; fungi<sup>19–22</sup> and an alga<sup>23</sup>, but not in higher plants. In the context of our results on the moss protonema, the situation in lower plants may indeed be different from that of higher plants. Based on available evidence, cyclic AMP seems to be a naturally-occurring regulator of growth and differentiation only in the lower plants.

**Table 3** Endogenous levels of cyclic AMP in wild-type and mutant protonema

Predominant cell type	Medium	% Chloronema	Cyclic AMP (pmol)	
			per g fresh weight	per mg protein
Wild type				
Chloronema	MM	100	56	2.05
	MM±1% glucose	100	44	2.23
Caulonema	MM±1 μM IAA	35	8	0.53
Mutant <i>pg-1</i>				
Caulonema	MM+1% glucose	25	5	0.53

Chloronema cells were grown in MM+1% glucose and collected in late exponential phase, whereas cultures of caulonema filaments were obtained by growing chloronema cells in MM+1  $\mu$ M IAA for 6 d. Mutant grew better in MM+1% glucose and cells were collected after 5 weeks. For cyclic AMP determination, cells were homogenised in 6%  $\text{Cl}_2\text{CCOOH}$  in a blender at 4 °C.  $^3\text{H}$ -cyclic AMP (3,000 c.p.m., specific activity  $20.8 \text{ Ci mmol}^{-1}$ ) was added as internal standard and homogenate was centrifuged at  $27,000g$  for 15 min. Pellet was saved for determination of protein<sup>15</sup> and cyclic AMP was determined in the supernatant. Supernatant was extracted five times with cold water-saturated diethyl ether and the resulting aqueous phase lyophilised after removal of ether. Dry material was dissolved in distilled water and chromatographed on BioRad Aminex MS cation exchange resin (mesh size 200–400). Cyclic AMP-containing fractions were freeze dried and chromatographed on thin-layer silica gel plates in *n*-butanol-methanol-ethylacetate-ammonia (7:3:4:4, v/v). Area corresponding to cyclic AMP was eluted with 50% ethanol and eluate lyophilised. Purified material was dissolved in distilled water and cyclic AMP determined using protein kinase assay as described by Kuo and Greengard<sup>16</sup> except that the source of protein kinase was rabbit skeletal muscle. Cyclic AMP levels in the samples of caulonema cells (wild type and *pg-1*) were three- to fivefold greater than the lowest amount of cyclic AMP detectable (0.5 pmol) in our assay conditions.

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## Adenylate cyclase activity and steroidogenesis in phenotypic revertants of an ACTH-insensitive adrenal tumour cell line

A LARGE body of evidence implicates adenylate cyclase and cyclic AMP as mediators of adrenocorticotrophic hormone (ACTH)-stimulated adrenal steroidogenesis (see ref. 1 for review). Recent observations, however, suggest that ACTH acts at separate sites to increase steroidogenesis and cyclic AMP accumulation, and that cyclic AMP may not be an important component of ACTH-stimulated steroidogenesis<sup>2-5</sup>. An ACTH-responsive adrenocortical tumour cell line<sup>6</sup> (clone Y1) and a derivative clone insensitive to ACTH<sup>7</sup> (clone Y6), have been used to examine the role of adenylate cyclase and cyclic AMP in adrenal steroidogenesis<sup>1,7</sup>. Y6 cells do not respond to ACTH with increased steroidogenesis but do respond to added cyclic AMP<sup>7</sup>. In addition, these cells have an adenylate cyclase system insensitive to ACTH, probably accounting for their variant phenotype<sup>1</sup>. Y6 cells, when grown as tumours, regain the ability to respond to ACTH with increased steroidogenesis, and continue to express the ACTH-sensitive phenotype after prolonged periods in culture and extensive dilution<sup>7</sup>. Y6 cells, after animal passage, provide a system of phenotypic revertants to examine the linkage of different biochemical events to ACTH insensitivity<sup>7,8</sup>. We have now examined adenylate cyclase activity in phenotypic revertants of Y6 cells induced by animal passage to assess the role of this enzyme in steroidogenesis. After animal passage, Y6 cells regained the ability to respond to ACTH both with increased steroidogenesis and with increased adenylate cyclase activity. These data indicate that the actions of ACTH on adenylate cyclase activity and steroidogenesis are closely linked.

Y6 cells were grown in monolayers or as tumours in isogenic mice (LAF<sub>1</sub>; Jackson Laboratories) as described previously<sup>7</sup>. For steroid assays, cells were incubated in growth medium containing 2.5 mM CaCl<sub>2</sub> and 0.1% trypsin inhibitor (from lima bean) for 1 h. At the end of the incubation periods steroids

were extracted from the growth medium in methylene chloride and measured against a corticosterone standard by fluorescence<sup>9</sup>. Adenylate cyclase activity was determined by measuring the conversion of labelled ATP to labelled cyclic AMP as described previously<sup>1</sup>. Labelled cyclic AMP was separated from other labelled compounds by chromatography on Dowex 50 and treatment with BaSO<sub>4</sub> (ref. 10). Cells were screened for aerobic and anaerobic bacteria to ensure that they were not contaminated. Mycoplasma infections were demonstrated by characteristic growth in broth and in agar<sup>11</sup>. Mycoplasma were determined by Dr P. Quinn, Hospital for Sick Children, Toronto.

Y6 cells infected with mycoplasma<sup>1</sup> tentatively identified as *M. arginini* (E. Bransome, personal communication) regained the ability to respond to ACTH with increased steroidogenesis when grown as tumours and plated as primary cultures (Table 1 and ref. 7). Concomitantly, the tumours and primary cultures recovered the ability to respond to ACTH with increased adenylate cyclase activity (Table 2). ACTH-sensitive adenylate cyclase activity was observed in intact cells, homogenates and membrane particles prepared from primary cultures of Y6 tumours (Table 2). Clonal isolates from primary cultures of Y6 cells after animal passage responded to ACTH and to cyclic AMP with increased steroidogenesis (Table 1). Homogenates and membrane particles prepared from these clonal isolates also responded to ACTH with increased adenylate cyclase activity (Table 2). Thus after animal passage, the same cell recovered the ACTH-sensitive phenotype both in steroidogenesis and adenylate cyclase activity. F<sup>-</sup> stimulated adenylate cyclase activity in preparations from primary cultures and to a greater extent in preparations from clonal isolates (Table 2). After animal passage, these cells no longer carried the mycoplasma infection.

Y6 cells cured of mycoplasma infection *in vitro* with kanamycin sulphate remained insensitive to ACTH<sup>1</sup>. After animal passage, the cells treated with kanamycin sulphate did not recover ACTH sensitivity with respect to steroidogenesis or adenylate cyclase activity (data not shown). When the cured cells were reinfected with mycoplasma and grown as tumours, they regained the ability to respond to ACTH with increased steroidogenesis (Table 1) and increased adenylate cyclase activity (Table 2). ACTH-responsive Y1 cells retained ACTH sensitivity when infected with mycoplasma and grown for several months (data not shown) or when treated with kanamycin sulphate<sup>1</sup>.

As demonstrated here, phenotypic reversion of Y6 cells requires the combination of mycoplasma infection and animal

Table 1 Steroidogenesis in Y6 cells after animal passage

Cell population	Steroids secreted (ng h <sup>-1</sup> per mg protein)		
	Unstimulated	+ACTH	+cyclic AMP
Experiment 1			
Primary culture	170 ± 15	710 ± 35	Not determined
Clone 1	80 ± 5	280 ± 30	215 ± 20
Clone 2	110 ± 5	580 ± 60	370 ± 15
Experiment 2			
Primary culture	200 ± 10	750 ± 50	Not determined

Y6 cells were grown as solid tumours in LAF<sub>1</sub> male mice and plated as primary monolayer cultures *in vitro*<sup>7</sup>. Clones were isolated by plating single cells from primary cultures in microwells for tissue culture. Where indicated, ACTH (ACTHar, Parke Davis) was added at 5 mU ml<sup>-1</sup> and cyclic AMP at 1 mM. In experiment 1 tumours were formed from Y6 cells infected with mycoplasma. Before animal passage, these cells secreted 180 ± 20 ng steroid per h per mg protein without ACTH; and 250 ± 15 ng steroid per h per mg protein with ACTH. In experiment 2 Y6 cells (approximately 10<sup>6</sup> cells per 75 cm<sup>2</sup> flask), cured of mycoplasma *in vitro* with kanamycin sulphate<sup>1</sup>, were reinfected by adding 0.1 ml of medium from mycoplasma-infected Y6 cells to 15 ml of culture medium. The infected medium was centrifuged at 200g to remove any floating cells before use. Cells were cultured for 2 weeks with mycoplasma titres reaching approximately 10<sup>7</sup> colony forming units in agar per ml of medium, and then injected into mice.

**Table 2** Cyclic AMP accumulation in Y6 cells after animal passage

Cell population	Cyclic AMP accumulation		
	Unstimulated	+ACTH	+F-
Experiment 1			
Solid tumour particles	30±5	110±5	—
Primary cultures			
Intact cells	2,030±430	4,900±160	—
Homogenates	100±10	950±70	2,330±120
Particles	65±5	165±10	3,130±210
Clone 1			
Homogenates	40±5	660±50	3,730±170
Particles	110±10	810±10	10,050±70
Clone 2			
Homogenates	80±10	270±10	4,480±70
Particles	110±5	240±10	7,780±160
Experiment 2			
Solid tumour particles	26±1	127±1	—

Cell populations were obtained for experiments 1 and 2 as described in Table 1. Adenylate cyclase in intact cells was determined by measuring the conversion of endogenous labelled ATP to labelled cyclic AMP as described previously<sup>1</sup> but without theophylline. ACTH was added to intact cells at 500 mU ml<sup>-1</sup>. The results for cyclic AMP accumulation in intact cells are expressed as c.p.m. per 30 min per mg protein ±s.e.m. Cell homogenates and membrane particles were prepared and assayed for enzyme activity as described previously<sup>1</sup>. ACTH (ACTHar, Parke Davis; 625 mU ml<sup>-1</sup>) and NaF (15 mM) were added where indicated. The results for cyclic AMP accumulation with homogenates and membrane particles are expressed as pmol per 10 min per mg protein ±s.e.m. Adenylate cyclase activity in homogenates of Y6 cells before animal passage was 30±5 pmol per 10 min per mg protein without ACTH; and 40±5 pmol per 10 min per mg protein with ACTH.

passage. Although the mechanism of restoration of ACTH sensitivity is not understood, these results suggest an unusual mechanism for the regulation of phenotypic expression. The possibility that this phenomenon is related to true genetic reversion remains open, since previous studies have demonstrated that mycoplasma can induce permanent phenotypic and karyotypic changes in cell cultures<sup>12</sup>.

Nevertheless, the concomitant recovery of ACTH-sensitive phenotypes in steroidogenesis and adenylate cyclase activity after animal passage demonstrate that the two events are closely linked. These observations indicate that the inability of Y6 cells to respond to ACTH with increased adenylate cyclase activity and with increased steroidogenesis is the result of a common defect, and suggest that at least one component of the ACTH-sensitive adenylate cyclase system is essential for the action of ACTH on adrenal steroidogenesis.

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## Inhibitory action of noradrenaline and cyclic AMP in explants of rat cerebellum

IZENTOPHORETICALLY applied noradrenaline (NA) produces sustained depression of spontaneous firing of rat cerebellar Purkinje cells<sup>1–3</sup> and this inhibition may be mediated by cyclic AMP<sup>1–3</sup>. This contention has been challenged by Godfraind and Pumain<sup>4</sup> as well as by Lake and Jordan<sup>5</sup> who failed to observe a depressant action of cyclic AMP. Because the results may be influenced by electrical current or pH artefacts, the kind of anaesthetics used or factors impeding the release of cyclic AMP from micropipettes<sup>7</sup> we have used cultured Purkinje cells from rat cerebellum<sup>8–10</sup> to test the hypothesis that cyclic AMP mediates the depressant action of NA on Purkinje cells. We found that high concentrations of cyclic AMP only slightly decreased the firing rate of Purkinje cells, but that low concentrations of inhibitors of phosphodiesterase strongly enhanced the inhibition induced by cyclic AMP and NA.

Explants of cerebellum were cultured from newborn rats by the roller tube technique<sup>11,12</sup>. All electrophysiological experiments were conducted during the third week in culture. Single unit spontaneous action potentials were recorded extracellularly from large nerve cells (presumably Purkinje cells) with microelectrodes filled with 2 M NaCl<sup>12</sup>. The drugs were added directly to Hanks' balanced salt solution which served also as control solution.

When the monoamine was added to the bathing fluid in low concentrations, NA, like isoproterenol<sup>8</sup>, altered the pattern of spontaneous discharges by selectively increasing the number of long interspike intervals. Higher concentrations (>10<sup>-5</sup> M) slightly decreased the firing rate and the cells stopped firing at a concentration varying between 10<sup>-4</sup>

**Table 1** Response of cultured Purkinje cells to various drugs applied in the bathing fluid

Substance	n	+	—	=
NA 10 <sup>-5</sup> M	14	29	14	57
NA 10 <sup>-4</sup> M	11	18	27	55
cyclic AMP 10 <sup>-4</sup> M	11	18	18	64
cyclic AMP 10 <sup>-3</sup> M	23	17	44	39
AMI 10 <sup>-4</sup> M	10	—	40	60
AMI 10 <sup>-3</sup> M	12	—	83	17
PAP 10 <sup>-6</sup> M	6	—	—	100
PAP 10 <sup>-5</sup> M	7	—	100	—
AMI 10 <sup>-4</sup> M + cyclic AMP 10 <sup>-4</sup> M	19	—	79	21
AMI 10 <sup>-4</sup> M + NA 10 <sup>-5</sup> M	10	—	70	30
AMI 10 <sup>-4</sup> M + NA 10 <sup>-4</sup> M	16	—	100	—
PAP 10 <sup>-6</sup> M + NA 10 <sup>-4</sup> M	6	—	100	—
cyclic AMP 10 <sup>-4</sup> M + NA 10 <sup>-4</sup> M	8	13	37	50
cyclic AMP 10 <sup>-3</sup> M + NA 10 <sup>-4</sup> M	8	—	75	25

Results are expressed as percentage of tested cells. n, Number of cells tested; +, excitation; —, inhibition; =, change in rate ≤ 10%; AMI, aminophylline; PAP, papaverine.

and 10<sup>-3</sup> M. The action of NA was, compared with other substances such as gamma aminobutyric acid<sup>8</sup> or glutamate<sup>10</sup>, slow in onset and only partially reversible. Only before the cessation of the activity did the amplitude of the spikes decrease.

Cyclic AMP had no effect at 10<sup>-4</sup> M. At 10<sup>-3</sup> M, the firing rate of 44% of the cells was decreased (Table 1), but no cells stopped firing. With monobutyl or dibutyl cyclic AMP, used on 14 cells (not shown in Table 1), there was no significant difference in response. Even in the absence of exogenous cyclic AMP, inhibitors of phosphodiesterase, believed to increase the intracellular cyclic AMP concentration, consistently depressed the firing of Purkinje cells (Fig. 1a). Papaverine was about 100 times more potent than aminophylline and caffeine in abolishing the



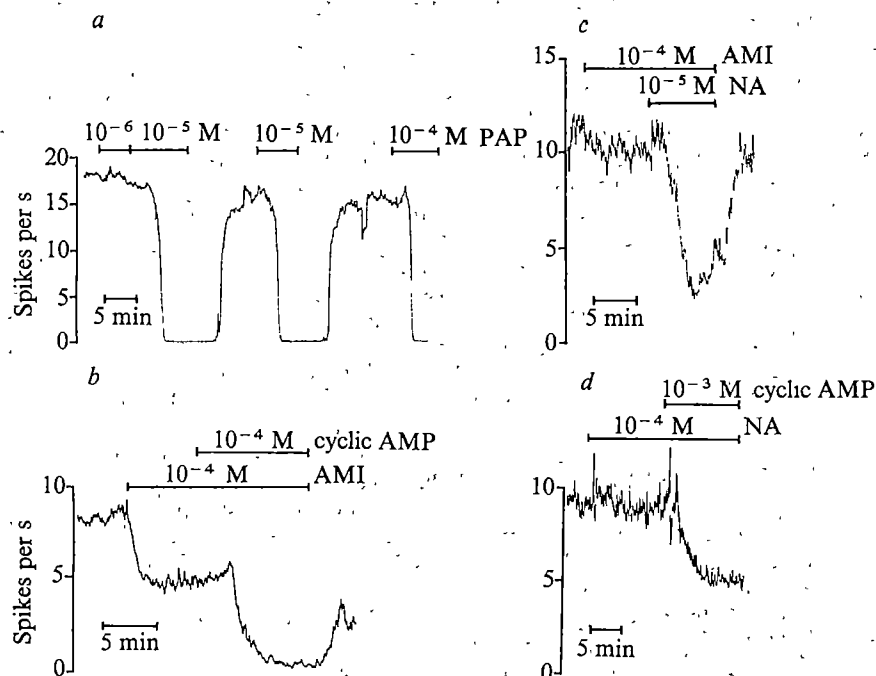


Fig. 1 Examples of the effects of various drugs on the rate of spontaneous discharge of Purkinje cells. *a*, Application of  $10^{-5}$  M papaverine abolished firing. After removal of the drug, the cells resumed firing. The effect of  $10^{-4}$  M papaverine was, however, only very slowly reversible. *b*, The inhibition induced by  $10^{-4}$  M aminophylline was enhanced by a subthreshold concentration of cyclic AMP. *c*, In the presence of aminophylline,  $10^{-5}$  M NA produced sustained depression of firing. In this cell, the rate of firing was not decreased by application of  $10^{-4}$  M aminophylline. *d*, High concentrations of NA and cyclic AMP slowed, but did not stop, firing of Purkinje cells.

spontaneous discharges ( $10^{-3}$  M of the latter were about equiactive with  $10^{-5}$  M papaverine).

The efficacy of various substances in potentiating the inhibitory effect is shown in Table 1 and examples of drug responses are illustrated in Fig. 1. The inhibition produced by  $10^{-4}$  M aminophylline was enhanced by a subthreshold concentration of cyclic AMP (Fig. 1*b*). On a cell which was virtually unaffected by  $10^{-4}$  M aminophylline, the addition of  $10^{-5}$  M NA caused sustained depression of firing (Fig. 1*c*). Surprisingly this inhibition was rather quickly reversed after removal of the drugs. Concomitant addition of  $10^{-4}$  M NA and  $10^{-3}$  M cyclic AMP inhibited Purkinje cells only to some degree (Fig. 1*d*), whereas most cells exposed to  $10^{-4}$  M NA and  $10^{-4}$  M aminophylline stopped firing.

Cultured Purkinje cells probably retain receptors for NA, and their activation could have been responsible for the inhibition in the presence of NA. The need for relatively high concentrations of NA to elicit an effect could be due to a relatively small number of intact receptors or, if the second messenger hypothesis of cyclic AMP is accepted, to high activity of phosphodiesterase. The latter might also be responsible for the failure of cyclic AMP to produce a clear, sustained depression of firing.

The fact that NA and inhibitors of phosphodiesterase produced similar electrophysiological results does not directly prove or disprove a link between their actions, but the hypothesis is strengthened because the action of NA was enhanced by inhibitors of phosphodiesterase. Substances that selectively inhibit brain adenylate cyclase might provide a means to determine whether cyclic AMP is the second messenger for NA in cerebellum. Lead was reported to inhibit selectively adenylate cyclase in rat cerebellum<sup>11</sup>. Unfortunately, application of lead ( $10^{-3}$ – $10^{-4}$  M) in our system led to ambiguous results, sometimes preventing, sometimes enhancing NA-induced inhibition.

In conclusion, phosphodiesterase activity seems to be high in cultured cerebellum. This might explain the weak effect of cyclic AMP (and possibly also of NA) on Purkinje cell firing. Inhibitors of phosphodiesterase strongly enhanced the inhibition produced by NA and cyclic AMP. The data reported support the hypothesis of Bloom *et al.*<sup>4,5,14</sup> that the intracellular level of cyclic AMP influences noradrenergic neurotransmission.

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## Allergic sensitisation of human lung fragments prevented by saturation of IgE binding sites

PASSIVELY sensitised chopped human lung fragments release mediators of immediate hypersensitivity when challenged with appropriate antigens<sup>1</sup>. Antigen triggers the release of mediators from mast cells or basophils by coupling of neighbouring surface IgE antibody molecules sharing the same antigen specificity<sup>2</sup>. Release of mediators should therefore be unlikely if specific IgE molecules are sparsely distributed on cell surfaces, as in normal non-allergic people; or if surface receptors are densely occupied by IgE molecules with other specificities. Humans harbouring certain parasites develop high IgE levels<sup>3</sup>, and epidemiological evidence suggests that allergic disorders such as hayfever or pollen asthma are rare in heavily parasitised populations<sup>4,5</sup>. It is not known whether IgE induced in man by parasite infestation is directed specifically against parasite antigens, or whether it represents nonspecific potentiation of IgE production against many different antigens, as demonstrated in the rat *Nippostrongylus* model<sup>6</sup>. Here we show that human lung fragments first exposed to IgE-rich serum from West African residents become resistant to further passive

Table 1 Effect of IgE-rich serum on subsequent passive sensitisation and challenge

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng per g. tissue, mean ± s.d.)
West African IgE-rich serum	Allergic serum	Pollen antigen	13	282 ± 92
Cord* serum	Allergic serum	Pollen antigen	13	2,316 ± 435
Cord serum	Allergic serum	Cord serum	13	171 ± 27
Cord serum	Cord serum	Pollen antigen	13	222 ± 33
West African IgE-rich serum	Cord serum	Pollen antigen	3	165 ± 54

\*Cord serum used in control experiments contained less than  $1 \text{ U ml}^{-1}$  of IgE

sensitisation with serum containing grass pollen-specific IgE. Normal tissue obtained from peripheral lung lobes removed at operation was finely chopped, filtered on gauze and washed three times in Krebs solution. Aliquots (200 mg) were incubated for 90 min at  $37^\circ\text{C}$  in pooled serum, diluted 1:100 in Krebs solution, from 8 West African subjects, residents in Manduar, The Gambia. The IgE level of this pool was  $6,454 \text{ U ml}^{-1}$ , measured by double antibody radioimmunoassay<sup>7</sup> ( $1 \text{ U} = \text{approximately } 2.4 \text{ ng}$  (ref. 8)). The treated aliquots of lung tissue were washed three times in Krebs solution and resuspended in serum, diluted 1:100, from a patient with hayfever. The total IgE of this second serum was only  $60 \text{ U ml}^{-1}$ , but high specific IgE antibody activity to Timothy grass pollen was demonstrated by the

a 73% blocking effect, comparing histamine release from blocked lung fragments with that from challenged control fragments exposed only to cord serum before passive sensitisation. Similar results were obtained when other allergic sera from different patients were used for passive sensitisation.

Table 2 shows the results of experiments designed to confirm that the blocking effect was related specifically to IgE antibody. All inhibitory activity was lost after heating the IgE-rich serum at  $55^\circ\text{C}$  for 30 min, enough to denature IgE but not other immunoglobulins. Furthermore, lung fragments exposed to unheated IgE-rich serum, although refractory to further passive sensitisation, released histamine readily on treatment with anti-IgE.

Table 2 Effect of heat treatment, and of challenge with anti-IgE

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng per g. tissue, mean ± s.d.)
Cord serum	Allergic serum	Pollen antigen	3	2,164 ± 184
Untreated IgE-rich serum	Allergic serum	Pollen antigen	3	238 ± 21
Heat-treated IgE-rich serum†*	Allergic serum	Pollen antigen	3	2,170 ± 240
—	IgE-rich serum	Anti-IgE†	2	2,998 ± 536
—	Allergic serum	Anti-IgE	2	3,435 ± 697
—	Cord serum	Anti-IgE	2	238 ± 32

\*Heated at  $55^\circ\text{C}$  for 30 min.

†Anti-IgE was prepared in a sheep immunised against N.D. myeloma IgE; the IgG fraction was separated from sheep serum by 3 ammonium sulphate precipitations, followed by DEAE-cellulose chromatography

radio-allergo-sorbent technique<sup>8</sup>. After overnight incubation at room temperature and a further 60 min at  $37^\circ\text{C}$  the lung fragments were washed and challenged with freeze-dried extract of Timothy grass pollen in Krebs solution ( $100 \mu\text{g ml}^{-1}$ ) for 15 min. Histamine released into the supernatant was measured by bioassay, using strips of guinea pig ileum bathed in Krebs solution at  $37^\circ\text{C}$  containing atropine sulphate  $60 \mu\text{g ml}^{-1}$ .

Table 1 shows the combined results of experiments in which previous exposure of lung fragments to IgE-rich serum effectively blocked subsequent passive sensitisation. There was some variation in histamine released by different lung specimens, but in no experiment was there less than

Finally, the effect of altering the experimental sequence was studied (Table 3). When lung was first passively sensitised with serum containing grass-pollen specific antibody, subsequent exposure to West African IgE-rich serum failed to interfere with histamine release after challenge. Nor was significant blocking found when lung fragments were exposed to grass-pollen specific antibody and IgE-rich serum simultaneously, in spite of a total IgE level 100-fold higher in the West African serum than in the allergic serum.

These results support the hypothesis that IgE receptors, numbering up to 100,000 per mast cell<sup>10</sup>, can be saturated, thus making the cell unresponsive to further attempts at passive sensitisation<sup>11</sup>. In this model system blocking was

Table 3 Effect of altering the experimental sequence

Preincubated with:	Lung fragments Sensitised with:	Challenged with:	No. of experiments	Mean histamine release (ng g <sup>-1</sup> tissue)
IgE-rich serum	Allergic serum	Pollen antigen	3	238 ± 21
Allergic serum	IgE-rich serum	Pollen antigen	3	2,348 ± 295
—	Mixed allergic serum and IgE-rich serum*	Pollen antigen	4	1,841 ± 173

\*The final dilution of both sera was 1:100.

achieved only if IgE-rich serum was added to lung in a separate step before passive sensitisation with allergic sera, results which are in keeping with the known high affinity binding of IgE to mast cell surface receptors. Mast cell blockade through saturation of IgE receptors is also suggested by the failure of patients with IgE myeloma to accept passive sensitisation<sup>12</sup>, and by animal experiments<sup>13,14</sup>. Now it is necessary to know whether IgE produced actively by parasite infection or by the administration of parasite derived antigen can displace IgE against environmental allergens already existing in an allergic patient.

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## Suppression of bacterially-induced hypersensitive reaction and phytoalexin accumulation in bean by phaseotoxin

PHASEOTOXIN is a trivial name given to an exotoxin produced by *Pseudomonas phaseolicola*, which causes halo-blight of beans<sup>1</sup>. Although the mechanism of chlorosis induction in susceptible hosts is unknown, evidence indicates that chlorosis is casually related to inhibition of ornithine carbamoyltransferase (OCT; EC 2.1.3.3) by phaseotoxin, a potent and specific inhibitor of the enzyme<sup>2</sup>. Phaseotoxin may also be involved in the host specificity of the pathogen. We previously reported that in infected resistant bean plants (but not in infected susceptible ones) phaseotoxin production is suppressed in spite of substantial bacterial multiplication<sup>3</sup>. Further, in resistant beans treated with phaseotoxin, a larger number of bacteria are found than in non-treated plants<sup>4</sup>. These observations indicate that phaseotoxin may suppress the hypersensitive reaction (HR) in resistant hosts. The object of this study was to investigate whether pretreatment of resistant cultivars of bean with phaseotoxin suppresses the HR response of the host and phytoalexin accumulation on subsequent inoculation with *P. phaseolicola*, and our results seem to support this.

The term HR is applied to an acute, localised response of plant tissues to inoculation with incompatible pathogens. In plants infected (or inoculated) with incompatible fungal pathogens, HR is accompanied by accumulation of phytoalexins, compounds which are toxic to the invading pathogens. There are only two examples of HR induction and phytoalexin accumulation in plants infected with incompatible bacterial pathogens<sup>5,6</sup>. The compounds which occur in soybeans inoculated with *P. glycinea* have been identified<sup>5</sup> and we have recently identified some of those which accumulate in beans infected with *P. phaseolicola*<sup>8</sup> and determined their toxicity to the pathogen.

Seedlings (6 d old) of a resistant bean cultivar were

allowed to take up nutrient solution plus phaseotoxin ( $4,200 \text{ U ml}^{-1}$ ) or nutrient solution alone (controls), in a growth chamber for 24 h. At the end of the incubation period the leaves of treated plants contained approximately  $1,300 \text{ U}$  of phaseotoxin per  $2 \text{ cm}^2$  leaf area. The plants were then replanted in vermiculite and the abaxial surfaces of their primary leaves inoculated with a suspension of *P. phaseolicola*. Leaf disks ( $2 \text{ cm}^2$ ) were removed every 24 h from the toxin-treated and control plants and their bacterial populations determined as previously described<sup>3</sup>.

In control plants the number of bacteria found per disk were  $7.1 \times 10^7$  and  $1.2 \times 10^8$  cells 2 and 4 d after inoculation, whereas in toxin-treated plants the bacterial numbers were  $6.7 \times 10^6$  and  $3.3 \times 10^{11}$  cells for the same periods (Fig. 1). After 3 d, the control plants responded with HR, exemplified by tissue silvering, collapse and browning. Leaves from treated plants were usually 0-5% smaller in area than the non toxin-treated control leaves and after 3 d showed water-soaked infection centres typical of the susceptible response. To determine if HR suppression by phaseotoxin is accompanied by suppression of phytoalexin accumulation, an identical experiment was performed except that only one leaf sample was taken. Leaves of control and toxin-treated plants were collected after the appearance of HR in control plants (3 d after inoculation) and their phytoalexin content determined by thin-layer chromatography<sup>9</sup>.

Chromatograms of extracts from control tissues showed several prominent fluorescing bands when exposed to ultraviolet or when sprayed with diazotised *p*-nitroaniline. In extracts of the toxin-treated tissues, however, only faint bands were seen. The known phytoalexin bands were removed, eluted from silica gel with 95% EtOH and rechromatographed in chloroform-EtOH (100:3). The chromatographically pure compounds were quantitated by spectrophotometry. In control tissues, quantities of phaseollin, phaseollidin, phaseollinisoflaven and kievitone were 12.21, 7.67, 9.14 and  $16.70 \mu\text{g}$  per g leaf tissue; whereas in toxin-treated tissues the quantities were 6.42, 3.04, 5.79 and  $12.86 \mu\text{g}$  per g fresh weight of the tissue respectively.

The demonstration of suppression of phytoalexin accumulation in resistant bean tissues by phaseotoxin offers a mechanistic explanation for the suppression of HR in such tissues. That such suppression results at concentrations of toxin which are detected in infected susceptible tissues

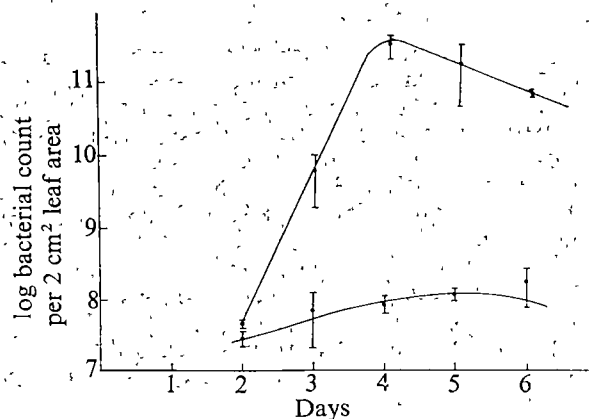


Fig. 1 Phaseotoxin treatment suppresses the resistance response in GN Nebraska 27 bean plants to *Pseudomonas phaseolicola*. Six-day-old seedlings were allowed to take up, through the roots, either quarter-strength nutrient solution or nutrient solution + phaseotoxin ( $4,200 \text{ U ml}^{-1}$ ) for 24 h in a growth chamber at  $27^\circ\text{C}$ . Leaf disks were taken at the end of the 24-h period and their phaseotoxin level determined ( $1,300 \text{ U per } 2 \text{ cm}^2$ ). The toxin-treated (○) and control (●) plants were then replanted and their primary leaves infiltrated with a cell suspension ( $10^7$  cells  $\text{ml}^{-1}$ ) of *P. phaseolicola* and incubated in a growth chamber (16 h light,  $24,000 \text{ lx}$ ) at  $24^\circ\text{C}$ . Leaf disks ( $2.0 \text{ cm}^2$ ) were removed daily from the leaves and their bacterial populations determined as described<sup>3</sup>. Bars represent s.e.m.

indicates that phaseotoxin is involved in the initial establishment of the pathogen in the susceptible tissues. That no phaseotoxin is detected in inoculated resistant tissues of bean further implicates the toxin in pathogenic establishment, and suggests that phaseotoxin production is suppressed in resistant tissues. Thus, it seems that the factor which determines bean resistance to *P. phaseolicola* has to do with suppression of elaboration of phaseotoxin.

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## Collaboration between *in vivo* responses to LD and SD antigens of major histocompatibility complex

STUDIES using mixed leukocyte culture (MLC) and cell mediated lympholysis (CML) tests, have suggested a possible differential role for LD and SD antigens associated with the major histocompatibility complex (MHC, H-2 in mouse) in allograft reactions<sup>1-3</sup>. LD differences are primarily responsible for stimulating the proliferative response in MLC; the SD antigens are the major targets in CML<sup>1</sup>. The presence of LD differences on the stimulating cell in MLC enhances the cytotoxic response against the SD antigens. The cellular basis for this potentiation of CML is that two separate subpopulations of T lymphocytes are differentially reactive to LD and SD antigens<sup>1-3</sup>. A proliferating helper cell responds primarily to LD differences whereas cytotoxic T lymphocytes are more strongly activated by the SD antigens. Lafferty *et al.*<sup>7</sup> and Talmage *et al.*<sup>10</sup> have shown that allogeneic thyroids transplanted under the kidney capsule are rejected less rapidly if they are first cultured *in vitro*. Injection of peritoneal exudate cells from the thyroid donor into the recipient speeds up rejection. They suggest that the peritoneal exudate cells sensitise the recipient and that the sensitised cells can invade and reject the thyroid. A further refinement in the interpretation of their data might suggest that either in addition to the mechanism they propose or as an alternative to it the peritoneal exudate cells provide an LD stimulus to the recipient's proliferating helper T cells, and that these cells either by themselves or through a secreted helper factor potentiate the development of cytotoxic T lymphocytes directed at the SD antigens on the graft. Our experiments reported here are consistent with this model.

The mouse strains we used are primarily strains AQR, B10.A and B10.T(6R). B10.A and B10.T(6R) are congenic on a B10 background. AQR carries a substantial portion of the B10 background but is not congenic with the other strains. Two mouse strains are said to be LD-different if they are identical for the two SD loci but differ for the

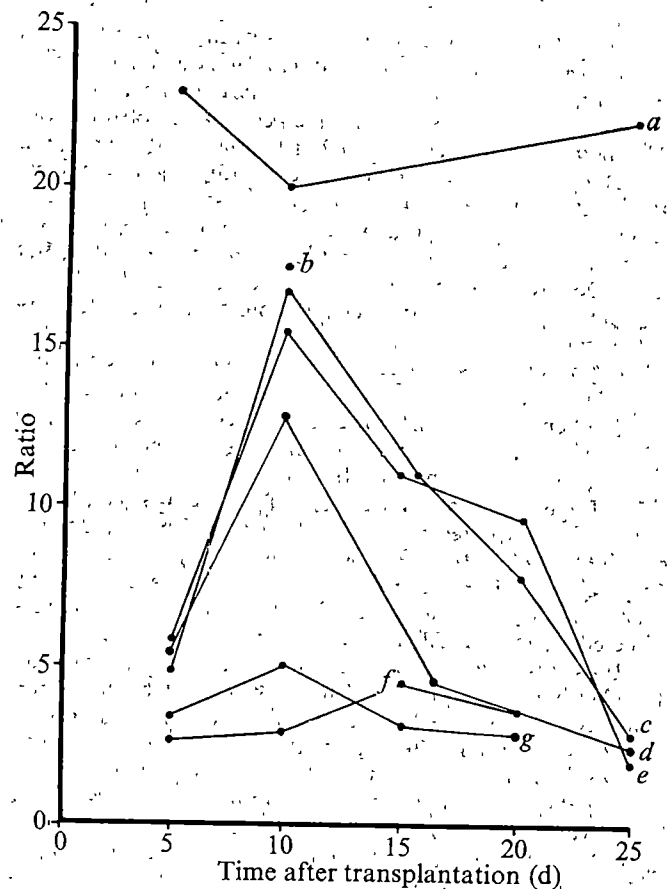


Fig. 1 Thyroid transplantation has been performed as described by Lafferty *et al.*<sup>6</sup> and Talmage *et al.*<sup>10</sup>. Twenty-four hours before removal of the kidneys 0.5  $\mu$ Ci of <sup>125</sup>I was injected intraperitoneally. Both kidneys of each animal were counted in a gamma counter. The results are expressed as a ratio of the counts of <sup>125</sup>I in the thyroid-containing kidney to those in the contralateral control kidney. All thyroids were examined histologically for signs of rejection; the results are consistent with those expected based on the ratio. We have injected  $1 \times 10^4$  lymph node cells (instead of peritoneal exudate cells) intraperitoneally into thyroid graft recipients. The H-2 complex can be divided into four regions, K, I, S and D. The SD loci are in the K and D regions; the strong LD locus is in I. The MHC genotypes of these strains are the following: AQR (qkdd), B10.A (kkdd) and 6R (qqdd), where the lower case letter in parentheses refers to the H-2 haplotype from which each region for a given strain is derived. Each point represents at least five animals. Comparative histological studies showed that thyroids with a ratio of 4 or lower reveal severe signs of rejection. The curves are keyed as follows: (recipient) (thyroid donor) (LNC donor). a, (AQR) (AQR) (none); b, (AQR) (AQR) (6R); c, (AQR) (B10.A) (AQR); d, (AQR) (B10.A) (B10.A); e, (AQR) (B10.A) (none); f, (AQR) (B10.A) (6R); g, (AQR) (B10.A) [(B10.A  $\times$  6R)(F<sub>1</sub>)].

strong LD locus in the I region, and SD-different if a difference exists for either the K and/or D region but they are identical for the H-2 I region. Thus, AQR and 6R are LD-different, and AQR and B10.A are SD-different (being identical for the strong LD locus (loci)). We have used lymph node cells (LNCs) instead of peritoneal exudate cells.

The results are presented in Fig. 1. B10.A thyroids transplanted into AQR recipients with or without injection of AQR LNCs show prolonged survival compared with grafts differing for both H-2 LD and SD factors which in our experiments never show ratios above 4 (data not shown) tested on day 5 and later<sup>6,10</sup>. Rejection is markedly more rapid when 6R LNCs are injected at the time of thyroid transplantation. Various controls are included in the figure. To rule out that the injected 6R cells themselves reject the thyroid rather than provide an LD stimulus to the recipient, in some experiments we have used [B10.A  $\times$  (6R)] F<sub>1</sub> LNCs instead of the 6R LNCs, similar enhancement of rejection was seen. (AQR  $\times$  B10) F<sub>1</sub> recipients gave results (not shown)



similar to the AQR recipients demonstrating that rejection is due to MHC differences (since AQR is not yet congenic with B10). Injection of B10.A LNCs into AQR recipients receiving a B10.A thyroid results in some acceleration of rejection. The potentiating effect is much less than that seen when 6R LNCs are injected and consistent with *in vitro* findings: B10.A stimulating cells not only induce a weak proliferative response in AQR responding cells, suggesting the presence of weak LD-like differences in the SD region of B10.A, but also lead to killing in CML<sup>7</sup>. Likewise, the fact that B10.A thyroids are rejected by AQR is consistent with these *in vitro* findings.

The most likely explanation of these findings is that LD-SD interaction similar to that seen *in vitro* can occur *in vivo*. When both an LD antigen on injected LNCs and SD differences on the B10.A thyroid are presented to the recipient's immune system, the B10.A grafts are rejected like LD+SD-different allografts. At the cellular level, this could be interpreted as follows. Injected LNCs from the LD-different 6R strain activate proliferating helper cells of the recipient. These cells, or soluble products secreted by them, amplify the response of the cytotoxic lymphocytes directed against the SD antigens in the thyroid.

B10.A thyroids are rejected in 20–25 d when no additional LNCs are injected. This delayed rejection is, presumably due to the absence of strong LD-like differences in this combination. We suggest that the prolonged survival of allogeneic cultured thyroids, at least in part, is likewise due to the removal of the LD stimulus in the passenger leukocytes.

AQR recipients recognise H-2<sup>I</sup> region associated antigens on the 6R LNCs; it could be argued that these antigens cross react with the SD antigens of B10.A recognised as foreign by AQR. *In vitro* data argue against this interpretation: AQR cells sensitised to 6R lyse 6R target cells but not B10.A<sup>11</sup>. We thus consider this a very unlikely explanation for our findings and suggest, instead, that LD-SD collaboration occurs *in vivo* as well as *in vitro*.

Davies and collaborators<sup>8,9</sup> have demonstrated that anti-I region (anti-Ia) antisera lead to prolongation of graft survival. Our studies suggest that, at least in part, I-region disparity is important in allograft rejection in terms of activating the T-helper system; they thus provide a potential mechanism for explaining the effect of the enhancing anti-Ia antisera as well as extrapolating the *in vitro* findings of LD-SD collaboration to the *in vivo* system. This work was supported by grants from the National Institutes of Health and the National Foundation—March of Dimes. H.W.S. is a recipient of a Max Kade postdoctoral research exchange grant. We thank Drs Kevin Lafferty and David Talmage for showing us the thyroid transplantation procedure and for discussion.

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## Association of high affinity stereospecific binding of <sup>3</sup>H-propranolol to cerebral membranes with $\beta$ adrenoceptors

*IN VITRO* binding of <sup>3</sup>H-catecholamines to plasma membranes from a variety of tissues has been reported<sup>1–4</sup>. In none of these studies, however, has the binding satisfied criteria necessary for identification of true  $\beta$  adrenoceptors (for example, stereospecificity and high affinity for potent  $\beta$ -adrenoceptor antagonists). On the other hand, studies using labelled  $\beta$ -adrenoceptor antagonists have demonstrated binding sites on erythrocyte ghosts and cardiac membranes that seem to satisfy fully the criteria associated with  $\beta$  adrenoceptors<sup>5–7</sup>.

The presence of  $\beta$  adrenoceptors in cerebral tissue has been confirmed by electrophysiological techniques<sup>8,9</sup>, and further characterisation of a central  $\beta$  adrenoceptor has come from biochemical approaches. For example, as is clearly the case in extracerebral tissue<sup>10</sup>,  $\beta$  adrenoceptors seem to mediate catecholamine-induced cyclic AMP formation in the brain<sup>11,12</sup>. Catecholamines have also been shown to interact with a  $\beta$  adrenoceptor and markedly stimulate cyclic AMP formation in neonate chick cerebral hemispheres<sup>13</sup>. Since it was felt that this tissue may represent a rich source of central  $\beta$  adrenoceptors, I have examined the characteristics of DL-<sup>3</sup>H-propranolol binding to membranes prepared from chick cerebral hemispheres.

Specific binding of DL-<sup>3</sup>H-propranolol to cerebral membrane fragments was saturable (Fig. 1) and half-maximal specific binding occurred at about 11 nM. On the other hand, nonspecific binding of DL-<sup>3</sup>H-propranolol (binding in the presence of 200  $\mu$ M isoprenaline) was not saturable over the concentration of propranolol examined. The amount of DL-<sup>3</sup>H-propranolol specifically bound at saturation provides an estimate of the number of binding sites on the membranes. The mean  $\pm$  s.e. for five experiments was 0.23  $\pm$  0.015 pmol per mg protein. DL-<sup>3</sup>H-propranolol bound rapidly and reversibly to cerebral membranes. Binding was 75% complete at 10 s and equilibrium was reached at 2 min, at which time the addition of unlabelled isoprenaline (200  $\mu$ M) resulted in a dissociation of bound DL-<sup>3</sup>H-propranolol with a half time of about 45 s.

The displacement of DL-<sup>3</sup>H-propranolol by non-radioactive stereoisomers, D- and L- and DL-propranolol is shown in Table 1. The D isomer was almost 100-fold less potent than the L isomer, and DL-pindolol proved to be the most potent  $\beta$ -adrenoceptor antagonist examined. The displacement of DL-<sup>3</sup>H-propranolol by catecholamines and salbutamol is also

Table 1 Effect of adrenergic antagonists and agonists on DL-<sup>3</sup>H-propranolol binding to crude synaptic membranes

Compound	Half-maximal inhibition of <sup>3</sup> H-propranolol binding
Antagonists	
DL-Propranolol	$9.5 \times 10^{-8}$ M
DL-Propranolol	$2.0 \times 10^{-8}$ M
D-Propranolol	$8.2 \times 10^{-7}$ M
DL-Pindolol	$6.1 \times 10^{-9}$ M
DL-Pronethalol	$1.4 \times 10^{-6}$ M
Agonists	
L-Isoprenaline	$1.2 \times 10^{-8}$ M
L-Adrenaline	$5.9 \times 10^{-6}$ M
L-Noradrenaline	$4.3 \times 10^{-5}$ M
Salbutamol	$1.1 \times 10^{-6}$ M
Dopamine	No binding at $10^{-4}$ M

For binding studies DL-<sup>3</sup>H-propranolol (15 nM) was incubated with various concentrations of compounds shown and membrane protein equivalent to 0.6–0.7 mg for 15 min at room temperature. Results are means of triplicate determinations from four separate experiments using at least six different concentrations of drugs indicated, s.e.m. all  $\leq 10\%$ .

shown in Table 1. Clearly the order of potency of the catecholamines was isoprenaline > adrenaline > noradrenaline. Salbutamol was about fourfold more potent than noradrenaline and dopamine was inactive at concentrations up to 100  $\mu$ M.

The results presented here demonstrate in a crude synaptic membrane preparation DL-<sup>3</sup>H-propranolol-binding sites that possess characteristics associated with true  $\beta$  adrenoceptors. Thus the binding is of high affinity, saturable, rapid, reversible and clearly stereospecific. Moreover, the cerebral membrane sites show high affinity for both  $\beta$ -adrenoceptor agonists and antagonists, and the order of potency of these compounds parallels their potency as agonists or antagonists on adenylate cyclase activity in various tissues<sup>5,7,15</sup>. The potency of isoprenaline, adrenaline, noradrenaline and salbutamol in displacing DL-<sup>3</sup>H-propranolol binding from chick cerebral hemisphere membrane sites closely approxi-

mates the potencies of these compounds in stimulating cyclic AMP formation in slices of cerebral hemispheres of this species<sup>13</sup>.

The data reported in cerebral membranes agree with extensive studies examining DL-<sup>3</sup>H-propranolol and L-<sup>3</sup>H-alprenolol binding to erythrocyte and cardiac membranes<sup>5,6</sup>, and suggests that the sites studied are indeed truly representative of  $\beta$  adrenoceptors. Earlier failures to detect stereospecific binding sites with DL-<sup>3</sup>H-propranolol in brain<sup>16</sup> and heart<sup>17</sup> probably relates to the use of high concentrations (100 nM) of DL-<sup>3</sup>H-propranolol rather than to the 'membrane' effects<sup>18</sup> of this drug.

The use of high specific activity  $\beta$ -adrenoceptor antagonists should make it possible to quantitate and further characterise this cerebral receptor. In addition it should allow for a more definite cellular localisation of the  $\beta$  adrenoceptor since their presence on both neurones and glia has been suggested<sup>9,19</sup>. Moreover, the use of DL-<sup>3</sup>H-propranolol should facilitate investigation of altered affinity and/or number of cerebral  $\beta$  adrenoceptors in conditions in which an apparent altered sensitivity to catecholamines has been demonstrated<sup>20,21</sup>.

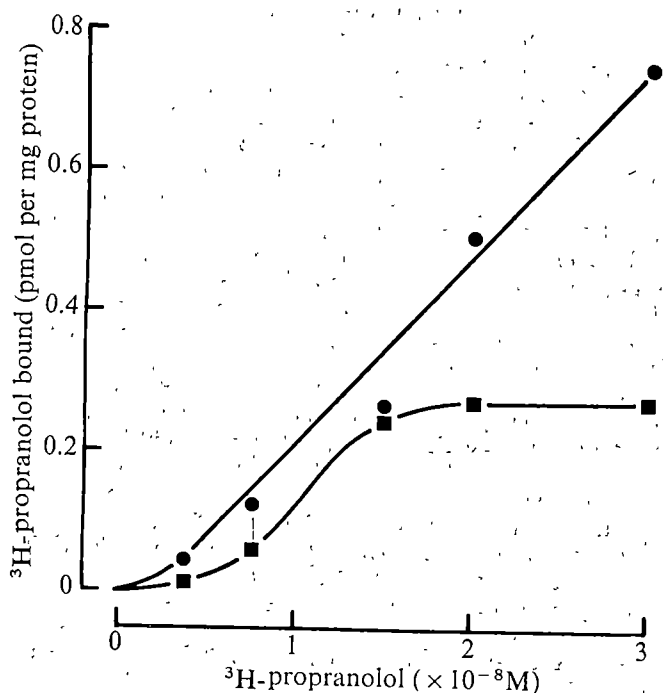
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**Fig. 1** Binding of DL-<sup>3</sup>H-propranolol to cerebral membranes. Male Ranger chicks (2–4 d old) were decapitated and their cerebral hemispheres homogenised in 20 volumes ice-cold 0.32 M sucrose and the homogenate centrifuged at 1,000g for 10 min. Resulting supernatant was centrifuged at 17,000g for 30 min to obtain a crude mitochondrial pellet. This pellet (P<sub>2</sub>) was lysed by resuspending in 20 volumes ice-cold water and briefly rehomogenising. The suspension was centrifuged at 10,000g for 20 min and the resulting pellet, a bilayer with a soft buffy upper layer washed carefully to collect this upper layer. Supernatant was centrifuged at 48,000g for 20 min and final membrane pellets stored in liquid nitrogen until use. Protein estimations by the method of Lowry *et al.*<sup>14</sup>. In the binding assay, membrane suspensions (0.5–1 mg protein) were incubated in all experiments except where indicated, with 15 nM DL-4n-<sup>3</sup>H-propranolol (specific activity 21 Ci mmol<sup>-1</sup>, Radiochemical Centre, Amersham), in buffer containing 50 mM Tris-HCl, pH 8.1, 15 mM MgCl<sub>2</sub> for 15 min at room temperature. Total volume of incubations 0.5 ml. Samples were filtered under pressure through Whatman glass-fibre circles (GFC) and immediately washed once with 10 ml buffer (filtering and washing required less than 6 s). Filters were dried and shaken in the cold with 5 ml Triton X100-toluene scintillator and radioactivity determined by liquid scintillation spectrometry. In every experiment 'nonspecific' binding was determined by measuring radioactivity obtained when incubations were carried out in presence of 200  $\mu$ M L-isoprenaline. Nonspecific DL-<sup>3</sup>H-propranolol binding (●) was subtracted from total radioactivity to obtain 'specific' binding (■). DL-<sup>3</sup>H-propranolol bound refers to 'specific' binding. At 15 nM DL-<sup>3</sup>H-propranolol, 'specific' binding was 40–50% total radioactivity. Results are means of triplicate determinations from five separate experiments; s.e.m. all < 10%.

## Synaptic transmission reversibly conditioned by single-gene mutation in *Drosophila melanogaster*

ONE way to advance current physiological and biochemical understanding of the mechanism of synaptic transmission at the molecular level is to alter synaptic function by means of single gene mutations. Among the various behavioural mutants of *Drosophila melanogaster*, those which express a mutant phenotype only in certain conditions and otherwise behave normally are of particular interest. If the mutant phenotype appears at the synapse, the mutant might enable a reversible modification of synaptic function by way of experimentally controlling the expression of the

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mutant phenotype. Such a mutant would provide a useful tool for the study of synaptic function.

Temperature-sensitive mutants which express their mutant phenotype only at a certain temperature are one class of conditional mutants. A single gene mutant of *D. melanogaster*, *shibire* temperature-sensitive (*shi<sup>ts1</sup>*), shows reversible paralysis at 29 °C, but behaves normally at 22 °C. Genetic and behavioural studies were previously done in Suzuki's laboratory<sup>1-4</sup>. We report here that the paralysis results primarily from a blockade of neuromuscular transmission.

The material used was *shi<sup>ts1</sup>*, one of the six alleles of the *shibire* locus. The mutant *shi<sup>ts1</sup>* not only shows a reversible temperature-sensitive paralysis but also expresses morphological changes on the epidermal structure if it is exposed to high temperature during development<sup>2</sup>. To avoid the latter effect, we have maintained the temperature during culture at 17 °C. Control wild-type Oregon-R flies were raised in the same conditions. Adult 4-d-old females of *shi<sup>ts1</sup>* and Oregon-R were used. For the physiological experiments, a fly was mounted in wax and immediately covered with saline solution<sup>5</sup> which was maintained at 19 °C by a thermoelectric unit placed under the bath. Artificial respiration was given through undamaged thoracic spiracles described previously<sup>6</sup>.

To confirm the temperature-sensitive paralysis, the mechanogram of the mesothoracic sterno-coxal muscle was recorded at various temperatures. For this purpose, the fly was placed ventral side up and the mesothoracic preepisternum was dissected out to expose the thoracic ganglion and the left mesothoracic leg nerve. The nerve was cut at about 20  $\mu$ m from the thoracic ganglion, and the distal cut end was sucked into a suction electrode for electrical stimulation. The left mesothoracic leg was cut at the coxal-trochanter joint, and a mechanoelectric transducer was attached to the proximal cut end of the leg. The other legs were held by wax at the femur to prevent disturbance to the record. The sterno-coxal muscle of *shi<sup>ts1</sup>* responded to a single electric pulse with a vigorous twitch, as shown in Fig. 1a (*shi<sup>ts1</sup>*) at 19 °C. When the temperature was increased to 27 °C, the magnitude of the response diminished. At 28 °C, only a small response was observed, and at 29 °C, the response was completely absent. The elimination of the mechanical response at 29 °C was

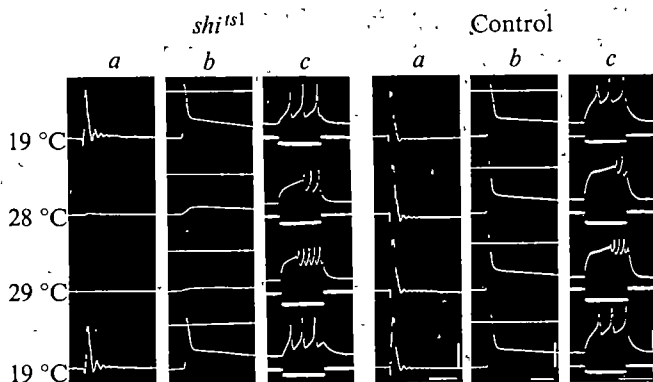


Fig. 1 Mechanical (a), junctional (b), and electrogenic (c) responses of mutant, *shi<sup>ts1</sup>*, and control, Oregon-R, flies. a, Tension developed in mesothoracic sterno-coxal muscle on stimulation of mesothoracic leg nerve. b, Junctional and action potentials of third dorsal longitudinal muscle fibre in response to stimulation of posterior dorsal mesothoracic nerve. Upper trace in each record shows reference potential level. c, Muscle membrane responses of third dorsal longitudinal muscle fibre for transmembrane depolarising current. Upper trace: potential recording; lower trace: current recording, outward, current downward. Temperatures are indicated at left of figures. Top 19 °C, beginning of experiment; bottom 19 °C, recovery after exposure to high temperature. Calibration for a: 2 mg, 250 ms; b: 50 mV, 10 ms; c: 50 mV,  $1 \times 10^{-7}$  A, 50 ms.

very specific and confirmed the previously reported behavioural results on paralysis<sup>3</sup>. When the temperature was lowered to 19 °C, the response returned almost completely. The change of temperatures from 19 to 29 and back to 19 °C was applied many times with consistently similar results, whereas the response from the control Oregon-R fly showed no appreciable change in mechanogram within this temperature range (Fig. 1). In several cases, the response of control flies remained unaffected even when the temperature was increased to 35 °C.

Our results indicated that the possible cause of paralysis is the excitability of the nerve, conduction of the impulse along the nerve, neuromuscular transmission, excitability of the muscle membrane, or contractile elements in the muscle fibre. From further studies, however, it seems that nerve excitability and conduction are not affected by the temperature change. To show this, the nerve was stimulated at the same location as described above, and nerve impulses were recorded at the femo-tibial joint as described previously<sup>5</sup>. The recorded nerve impulses from *shi<sup>ts1</sup>* showed no appreciable diminution at 29 °C, nor did those from the control fly Oregon-R.

For further study of the neuromuscular properties of these mutants, we used the dorsal longitudinal flight muscle because a fibre of this muscle was known to be innervated by a single excitatory neurone<sup>7</sup>. For this purpose, a fly was mounted laterally and dissected as described previously<sup>7</sup>. The entire lateral surface of the dorsal longitudinal muscle and the thoracic ganglion was exposed to saline solution by making an opening at the lateral cuticle and removing the dorso-ventral and tergo-trochanter muscles. The posterior dorsal mesothoracic nerve innervating the exposed dorsal longitudinal muscle was cut about 50  $\mu$ m from the ganglion. The distal cut end was stimulated with a suction electrode. Intracellular recordings were made from the third muscle fibre. At 19 °C, both control Oregon-R and *shi<sup>ts1</sup>* flies showed membrane potentials of -88 to -93 mV. The preparations showing resting potentials less negative than this range were discarded. In the remaining preparations, a single square current pulse of 0.1 ms was applied to the nerve every second, and the muscle membrane response was recorded continuously as the temperature was changed.

The muscle membrane response of *shi<sup>ts1</sup>* is shown in Fig. 1b (*shi<sup>ts1</sup>*). The top record of Fig. 1b (*shi<sup>ts1</sup>*) shows the response at 19 °C. The response consisted of a rapidly increasing and overshooting action potential followed by the remaining slowly decreasing junction potential. When the temperature was raised, the action potential began to fail, leaving a junction potential of diminished size. At 28 °C only the junction potential was recorded. At 29 °C the junction potential was markedly reduced; and when the preparation was kept at 29 °C for longer than 5 min, no trace of junction potential was observed. Lowering the temperature to 19 °C restored the response completely. The effects of these changes of temperature were consistent and repeatable. During the change of temperature, the resting membrane potential showed no appreciable change and stayed at -90 mV. The response of the muscle membrane on stimulation of the nerve of the control fly Oregon-R is shown in Fig. 1b. The response at 19 °C was similar to that of *shi<sup>ts1</sup>*. When the temperature was raised, the time course of the action potential became faster but never failed to respond even up to 35 °C. The results suggest that the possible cause of the paralysis in *shi<sup>ts1</sup>* could be at the neuromuscular junction or more distal part of the system, possibly the muscle fibre membrane.

The excitability of the muscle membrane was tested by passing electric current through an intracellular microelectrode while observing the response by another intracellular electrode. As shown in Fig. 1c, the muscle membrane of both *shi<sup>ts1</sup>* and Oregon-R flies responded with a train of

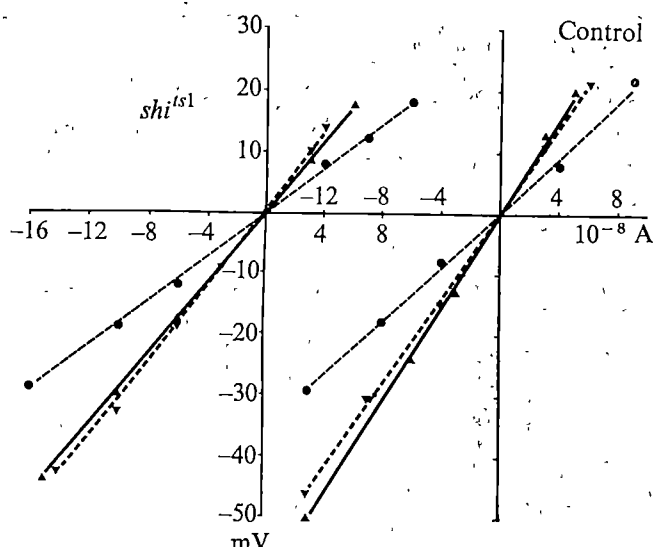


Fig. 2 Current-voltage relationship at different temperatures obtained from mutant, *shi<sup>ts1</sup>*, and control, Oregon-R, flies.  $\Delta$ , 19 °C, beginning;  $\bullet$ , 29 °C;  $\nabla$ , 19 °C, recovery. Depolarisation and outward current are plotted in positive direction on y and x axes, respectively. Resting potential located at origin.

action potentials after local depolarisation. At high temperatures, the amplitude of the action potential became smaller and a larger depolarising current was required to initiate action potentials. All of these effects of temperature, however, appeared similarly in both *shi<sup>ts1</sup>* and Oregon-R flies. The current-voltage relationship of the muscle fibre membrane was analysed by applying hyperpolarising and depolarising currents to the fibre. As shown in Fig. 2, the input conductance increased by a factor of about 1.7 when the temperature was raised from 19 to 29 °C. The input conductance returned essentially to the original level when the temperature was lowered. These changes were common to both *shi<sup>ts1</sup>* and Oregon-R flies. Thus, the capacity of electrogenesis of the muscle membrane was found to be unaffected by the *shi<sup>ts1</sup>* gene.

So far, we have not investigated the effect of this mutation on the excitation-contraction coupling mechanism or the contractile protein. Therefore, we cannot eliminate the possibility that these and related properties necessary for the contraction of the muscle are affected by mutation. If the neural information carried by the nerve fails to be transmitted, however, to the muscle membrane at the neuromuscular junction, as shown by *shi<sup>ts1</sup>* at high temperature, the failure of muscle contraction is independent of the state of the contractile mechanism. Thus, the major cause of paralysis induced by the *shi<sup>ts1</sup>* gene is the blockade of neuromuscular transmission.

We have not determined whether the failure of transmission is presynaptic or postsynaptic. Kelly and Suzuki<sup>8</sup>, however, reported that the loss of the on-off transient of electroretinogram of *shi<sup>ts1</sup>* at high temperature was attributable to both pre- and postsynaptic defects. It is likely that not only the neuromuscular junction but other synapses are affected by the *shi<sup>ts1</sup>* gene. At the presynaptic terminal, if the potential-dependent calcium permeability<sup>9</sup> is affected, the transmitter release mechanism may be altered. The action potential of the muscle fibre is produced by the potential-dependent increase in calcium permeability (unpublished data on *Drosophila* by the present authors and also on *Sarcophaga* by J. B. Patlak). Note that the calcium permeability of the muscle fibre membrane is not affected in *shi<sup>ts1</sup>*.

At the postsynaptic membrane, one possibility is the direct effect on the receptor protein; it is known that the change of a single amino acid in a polypeptide could result in altering the temperature sensitivity of the protein<sup>10</sup>.

Membrane permeability changes at the subsynaptic membrane also should be investigated. Although we have not determined these points, the present results are the first to reveal that a temperature-conditioned neuromuscular transmission can be induced by a single gene mutation. Siddiqui and Benzer reported similar results (unpublished) with another allele of *shi<sup>ts</sup>*.

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## Ca<sup>2+</sup> influx across the excitable membrane of behavioural mutants of *Paramecium*

THE ciliate protozoan *Paramecium* has an excitable surface membrane, which depolarises in response to a sudden increase in the Na<sup>+</sup> or K<sup>+</sup> concentration of the medium. An influx of Ca<sup>2+</sup> during depolarisation raises the internal Ca<sup>2+</sup> concentration, which in turn causes the cilia to reverse their direction of beating, and the cells to swim backwards<sup>1</sup>. The swimming behaviour of *Paramecium* is therefore a direct and readily observable correlate of the electrophysiological state of the membrane.

We have developed (unpublished results) a method of measuring <sup>45</sup>Ca<sup>2+</sup> influx and shown that in wild-type cells, the rate of <sup>45</sup>Ca<sup>2+</sup> influx increased five- to tenfold when 10 mM Na<sup>+</sup> or K<sup>+</sup> was added. This stimulated influx correlated with the observed backward swimming which in turn is correlated with Ca<sup>2+</sup> movement down an electrochemical gradient through a Ca<sup>2+</sup> "gate". This gating mechanism is a major component of membrane excitability in *Paramecium*<sup>1,2</sup>.

By selecting for behavioural defects, Kung *et al.* isolated several classes of mutant *paramecia* defective in various components of membrane excitability<sup>2</sup>. We have measured <sup>45</sup>Ca<sup>2+</sup> influx in all the major classes of mutants. Cells that showed no backward movement in Na<sup>+</sup> or K<sup>+</sup>, also showed little Na<sup>+</sup>- or K<sup>+</sup>-stimulated Ca<sup>2+</sup> influx.

Ca<sup>2+</sup> influx was measured essentially as before (to be described elsewhere). A low Ca<sup>2+</sup> concentration was used to minimise Ca<sup>2+</sup> binding, and the assay was performed at 0 °C to inhibit an active Ca<sup>2+</sup> extrusion. Cells were separated from the assay solution by centrifugation in a Pasteur pipette with one end sealed<sup>3</sup>.

Both behaviour and cell survival at 0 °C were examined. In the assay solution (legend Table 1) at 0 °C the cells



Table 1. Rate of  $\text{Ca}^{2+}$  influx in behavioural mutants of *Paramecium*

Table 1. Rate of $\text{Ca}^{2+}$ influx in behavioural mutants of <i>Caenorhabditis elegans</i>									
Phenotype	Genotype and strain	Growth temp. ( $^{\circ}\text{C}$ )	Rate of $\text{Ca}^{2+}$ influx				Backward swimming in		
			Control	NaCl (mM)		KCl (mM)		$\text{Na}^{+}$	$\text{K}^{+}$
Wild type	(51s)	24-26	3.0	8.5	11.0	12.3	16.0	+	++
		24-26	1.7	6.2	11.5	15.0	19.0		
		24-26	1.1	1.2	4.0	6.6	13.4		
		35	3.0	8.0	9.2	11.0	10.5	+	++
		35	1.2	4.3	4.5	4.5	6.6		
Pawn	<i>pwA</i> (d4-94)	24-26	1.1	1.7	2.5	1.1	3.3	—	—
		24-26	2.0	2.7	2.8	2.1	5.4		
	<i>ts-pwA</i> <sup>2</sup> (d4-133)	24-26	1.0	6.1	7.6	11.0	16.3	+	++
		24-26	2.6	6.5	6.3	7.4	11.1		
		35	2.3	1.7	2.1	2.6	2.6	—	—
	<i>pwB</i> (d4-95)	35	0.8	0.7	1.0	1.1	0.6		
		24-26	1.2	1.5	2.0	0.8	1.4	—	—
	<i>ts-pwC</i> (d4-131)	24-26	1.1	1.0	1.0	1.0	1.0		
		24-26	1.1	2.2	2.1	3.7	5.9	+	++
		24-26	1.3	2.6	3.0	2.5	4.0		
		35	2.3	1.3	1.7	1.7	2.5	—	—
		35	1.0	0.5	1.0	1.0	1.2		
Paranoiac	<i>PaA</i> (d4-90)	24-26	2.7	19.3	16.8	18.7	15.2	++	++
		24-26	1.7	9.2	8.9	9.0	9.0		
	<i>PaC</i> (d4-150)	24-26	1.7	8.8	12.4	12.5	9.3	++	++
		24-26	1.0	13.0	13.5	7.0	7.2		
	<i>fna</i> <sup>P</sup> (d4-149)	24-26	1.5	3.5	4.0	4.2	7.5	++	++
		24-26	1.5	3.5	4.0	5.5	13.0		
Fast-1	<i>fA</i> (d4-98)	24-26	2.4	7.7	5.7	7.7	8.7	+	++
		24-26	2.6	5.8	5.6	8.2	11.3		
Fast-2	<i>fna</i> (d4-91)	24-26	2.0	2.2	1.7	10.5	10.1	—	++
		24-26	2.1	2.0	2.0	12.4	13.5		
TEA	<i>TEA</i> (d4-152)	24-26	2.0	7.0	8.5	6.0	9.0	+	++
		24-26	1.2	3.2	3.6	7.0	9.0		

Influx was measured as nmol  $\text{Ca}^{2+}$  per 10 min per mg protein. Mutants were derived from 51s and were homozygous for mutations at the designated gene loci. Strain numbers are given in parentheses. —, No backward swimming observed; +, cells show repeated backward jerks; ++, cells swam backwards continuously.

*Paramecium aurelia* (syngen 4) was grown in cerophyl medium<sup>7</sup> (supplemented with  $\beta$ -sitosterol ( $1 \text{ mg l}^{-1}$ ) and inoculated 16 h previously with *Enterobacter cloacae*, the food source). Cells were collected by centrifugation at mid to late logarithmic phase, washed once in the assay solution and transferred into the assay solution. The assay solution consisted of: 10% v/v cerophyl medium (prepared without  $\text{Na}_2\text{HPO}_4$ ), 1 mM HEPES, 50  $\mu\text{M}$   $\text{CaCl}_2$ , 20  $\mu\text{M}$  EDTA and Tris base to pH 7.0. This solution contained 40  $\mu\text{M}$  free  $\text{Ca}^{2+}$ . Cells (30–90,000 per ml) were left in the assay solution for 30 min at room temperature. Then, the cells were placed on ice for 20 min before  $^{45}\text{Ca}^{2+}$  influx was measured and were left on ice for the rest of the experiment.  $^{45}\text{CaCl}_2$  (New England Nuclear, titrated to a phenol red endpoint with Tris base) was added to a specific activity of  $\text{Ca}^{2+}$  of 1,000 c.p.m.  $\text{nmol}^{-1}$ . Duplicate samples of cells were collected by layering 0.5 ml of the cell suspension over 1.0 ml of wash solution (assay solution with 1% sucrose), in a chilled, siliconised Pasteur pipette centrifuge tube. The tube was centrifuged for 0.5–1.0 min at 500g in an oil testing centrifuge and the tip containing the cell pellet was broken off. The tips were crushed and counted by liquid scintillation in a Triton-toluene mixture<sup>8</sup>. Uptake was approximately linear over the first 20 min. Rates were determined at 10 and 20 min and the average was taken. A large amount (30–50%) of the total  $\text{Ca}^{2+}$  in the assay mixture had been taken up after 30 min at  $0^{\circ}\text{C}$  when cells were stimulated by 10–15 mM KCl or NaCl. When the number of cells in the assay was decreased, however, we found no appreciable difference in uptake rates, expressed as nmol  $\text{Ca}^{2+}$  per 10 min per mg protein.

swam; though more slowly than at  $25^{\circ}\text{C}$ , for at least 1 h. When  $\text{Na}^{+}$  or  $\text{K}^{+}$  (10–15 mM) was added, 20–80% of the cells swam very slowly or stopped after 10–30 min. These cells swelled slightly, yet lysis was never observed. Naitoh<sup>4</sup> has shown that cilia cease to beat when the intracellular  $\text{Ca}^{2+}$  concentration rises above 0.1 mM. Cells stopped swimming at the end of the experiment only when  $\text{Na}^{+}$  or  $\text{K}^{+}$  was added, at a final concentration of 10 mM or higher. Pawn mutants, which did not show stimulated  $\text{Ca}^{2+}$  influx, also did not stop swimming in the presence of  $\text{Na}^{+}$  or  $\text{K}^{+}$ . For these reasons, we believe that the poor condition of the cells at the end of the experiment is a result of a large accumulation of intracellular  $\text{Ca}^{2+}$  in our assay conditions, which were chosen to prevent normal  $\text{Ca}^{2+}$  expulsion. The increased intracellular  $\text{Ca}^{2+}$  concentration apparently affects cell motility and shape.

Wild-type cells at  $0^{\circ}\text{C}$  swam backwards or showed repeated backward jerks in solutions containing  $\text{Na}^{+}$  or  $\text{K}^{+}$ , as they did at room temperature. The response of mutant cells to  $\text{Na}^{+}$  and  $\text{K}^{+}$  at  $0^{\circ}\text{C}$  was also qualitatively the same

as the response at room temperature. In the absence of  $\text{Na}^{+}$  or  $\text{K}^{+}$ , spontaneous avoiding reactions were more frequent at  $0^{\circ}\text{C}$  than at room temperature. These spontaneous reversals were probably due to an abnormal accumulation of  $\text{Ca}^{2+}$ , which occurred when the  $\text{Ca}^{2+}$  efflux system was inhibited by low temperature.

$\text{Ca}^{2+}$  influx in wild-type cells was stimulated by both  $\text{Na}^{+}$  and  $\text{K}^{+}$ , with  $\text{K}^{+}$  more effective than  $\text{Na}^{+}$  (Table 1). This trend was observed in each of approximately 20 experiments with wild-type cells. The absolute rate of stimulated  $\text{Ca}^{2+}$  influx varied over a range of 6–20 nmol per mg protein per 10 min in different experiments. This variability may be due to differences in the nutritional state of the cells which are difficult to control. In starved or slowly growing cells,  $\text{Na}^{+}$  generally stimulated  $\text{Ca}^{2+}$  influx only weakly.

$\text{Ca}^{2+}$  influxes were measured for 10 behavioural mutants of *Paramecium*, each with a different, single gene mutation. These mutants belong to the following classes: pawns, paranoiacs, fast-1, fast-2, and TEA (tetraethyl ammonium)-insensitive.

Pawn mutants always swim forward and do not reverse their swimming direction in  $\text{Na}^+$  or  $\text{K}^+$ . This behaviour has been attributed to a mutation in a voltage-sensitive  $\text{Ca}^{2+}$  gate, which cannot open properly to allow  $\text{Ca}^{2+}$  down its electrochemical gradient<sup>2</sup>.  $\text{Na}^+$  and  $\text{K}^+$  failed to stimulate  $\text{Ca}^{2+}$  influx in the unconditional pawn, *pwB*, and in the temperature-sensitive pawns, *pwA*<sup>2</sup>, and *pwC* grown at 35 °C (Table 1).  $\text{Ca}^{2+}$  influx in the pawn mutant *PwA* was weakly stimulated by  $\text{Na}^+$  and  $\text{K}^+$ . This mutant is somewhat leaky

(C. Kung, personal communication). Temperature-sensitive pawns exhibit wild-type behaviour when grown at room temperature and pawn behaviour when grown at 35 °C (ref. 6). Figure 1 shows the effects of  $\text{Na}^+$  and  $\text{K}^+$  on  $\text{Ca}^{2+}$  influx in wild-type and temperature-sensitive pawns grown at 24–26 °C and at 35 °C. Temperature-sensitive pawns require 2–4 h of growth at the permissive temperature to shift from pawn to wild-type behaviour<sup>3</sup>, so cells grown at 35 °C could not have shifted into a new phenotype during the assay at 0 °C.

The pawn mutant *pwC* (d4-131) has reproducibly lower rates of  $\text{Ca}^{2+}$  influx when grown at the permissive temperature (24–26 °C) (Table 1) and may therefore be somewhat abnormal even when grown at the permissive temperature. Kung has presented genetic evidence that one or both of the gene products of *pwA*<sup>1</sup> and *pwC* do not function normally even at the permissive temperature. Although both *pwC* and *pwA*<sup>1</sup> are temperature-sensitive pawns, the double mutant *pwA*<sup>1</sup>/*pwC* is temperature independent<sup>3,6</sup>.

Paranoiac mutants swim backwards for abnormally long periods in  $\text{Na}^+$ , yet swim backwards normally in  $\text{K}^+$  (ref. 2). The biochemical nature of the defect is not known.  $\text{Na}^+$  was as effective as  $\text{K}^+$  in triggering  $\text{Ca}^{2+}$  influx in the paranoiac *PaA*, and in another paranoiac strain, *PaC*,  $\text{Na}^+$  stimulated  $\text{Ca}^{2+}$  influx even more effectively than  $\text{K}^+$  (Fig. 1 and Table 1). The rates of  $\text{Ca}^{2+}$  influx in the paranoiacs were correlated with the behaviour of paranoiacs, since  $\text{Na}^+$ -stimulation caused increases in both the duration of backward swimming and the rate of  $\text{Ca}^{2+}$  influx.

Fast-1 and fast-2 mutants swim faster than normal cells. Fast-1 responds normally to  $\text{Na}^+$  and  $\text{K}^+$ , while fast-2 reverses only in  $\text{K}^+$  but not in  $\text{Na}^+$  (ref. 2). Fast-2 cells seem to have an abnormally large resting  $\text{K}^+$  permeability (Y. Satow, and C. Kung, personal communication).  $\text{Ca}^{2+}$  influx in fast-1 cells was stimulated normally by  $\text{Na}^+$  and  $\text{K}^+$ , while only  $\text{K}^+$  stimulated  $\text{Ca}^{2+}$  influx in fast-2 (Table 1 and Fig. 1). Again, there was a direct correlation between  $\text{Ca}^{2+}$  influx and ciliary reversal.

The mutant *fna*<sup>p</sup> behaves as a paranoiac, but the mutation is located in the fast-2 gene (*fna*) (J. Van Houten, S.-Y. Chang, and C. Kung, personal communication).  $\text{Na}^+$  stimulated  $\text{Ca}^{2+}$  influx in this cell line to a rate similar to that in wild type (that is, intermediate between fast-2 and paranoiac).  $\text{K}^+$ -stimulated  $\text{Ca}^{2+}$  influx normally in the *fna*<sup>p</sup> mutant (Table 1).

The mutant *TEA*<sup>-</sup> swims forward instead of reversing in the presence of TEA (which causes backward jerking in wild-type cells). The *TEA*<sup>-</sup> mutant responds almost normally to  $\text{Na}^+$  and  $\text{K}^+$  (ref. 2), but it apparently has a membrane defect resulting in a low resting resistance, most likely due to an increase in conductance to potassium (Y. Katow, and C. Kung, personal communication). *TEA*<sup>-</sup> cells had normal  $\text{Na}^+$ - and  $\text{K}^+$ -stimulated  $\text{Ca}^{2+}$  influx (Table 1).

There was an excellent correlation for all the cell lines studied between an increased  $\text{Ca}^{2+}$  influx triggered by  $\text{Na}^+$  and  $\text{K}^+$  and backward swimming stimulated by  $\text{Na}^+$  and  $\text{K}^+$ . These results further document that the three pawn genes, *pwA*, *pwB* and *pwC* are involved with the  $\text{Ca}^{2+}$  "gate", since these are the only mutants that show little  $\text{Ca}^{2+}$  influx in both  $\text{Na}^+$  and  $\text{K}^+$  solution. Our results suggest that fast-2 and paranoiac mutants, which show altered behaviour in  $\text{Na}^+$ , have normal  $\text{Ca}^{2+}$  "gates", since they show normal  $\text{Ca}^{2+}$  influxes in  $\text{K}^+$  solution. The abnormal response to  $\text{Na}^+$  in these strains is apparently the result of other factors. The variation between wild type, paranoiac and fast-2 phenotypes could be explained by postulating separate  $\text{Na}^+$ - and  $\text{K}^+$ -triggered  $\text{Ca}^{2+}$  "gates". We consider separate gates unlikely, since a single mutation in pawns can eliminate all  $\text{Ca}^{2+}$ -mediated depolarisations<sup>2</sup>.

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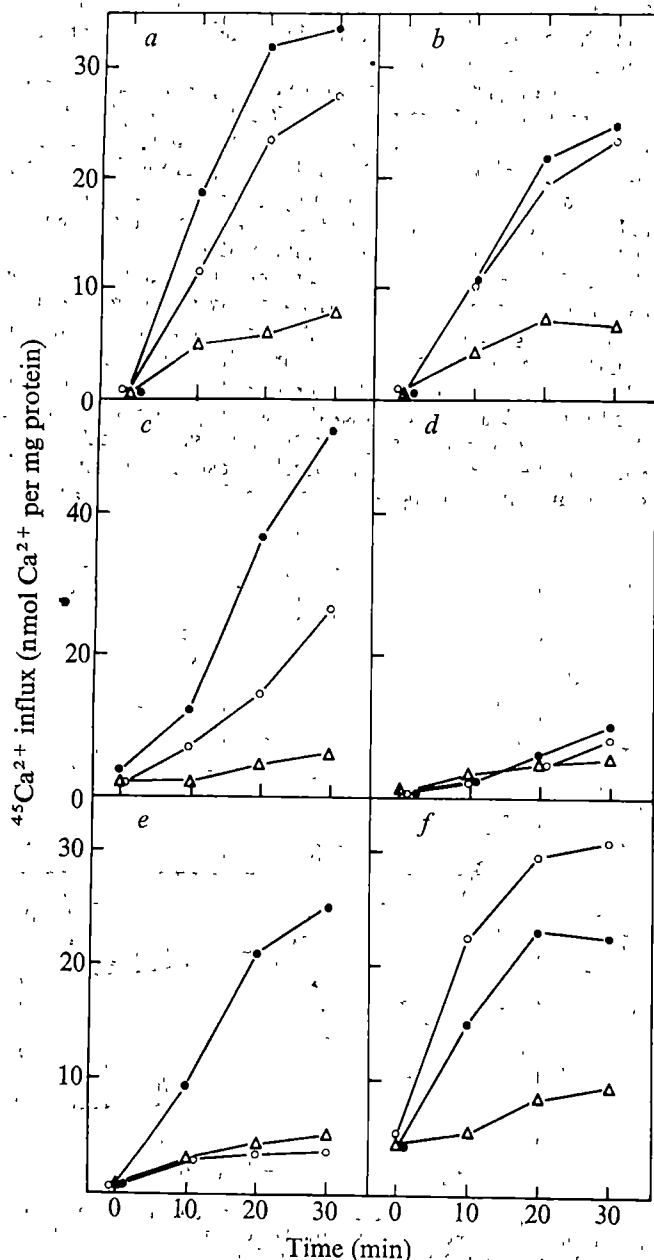


Fig. 1  $^{45}\text{Ca}^{2+}$  influx in wild type and behavioural mutants of *Paramecium*.  $\text{Ca}^{2+}$  influx was assayed as described in the legend to Table 1. Cell cultures were grown at 24–26 °C as described in the legend to Table 1. Cells were fed with fresh cerophyl medium which had been inoculated 12 h previously with *Enterobacter cloacae*. Half of the culture was left at 24–26 °C and the other placed at 35 °C.  $\text{Ca}^{2+}$  influx was assayed after 20 h or more. Each point represents the average of duplicate samples. The difference between duplicate values averaged 0.7 nmol  $\text{Ca}^{2+}$  per mg protein, for 40 separate points.  $\text{Ca}^{2+}$  influx was measured with 15 mM KCl (●), 15 mM NaCl (○), and the control (no addition) (△). a, wild type (25 °C); b, wild type (35 °C); c, *ts-pwA*<sup>2</sup> (d4-133, 25 °C); d, *ts-pwA*<sup>2</sup> (d4-133, 35 °C); e, *fna* (d4-91, 25 °C); f, *PaC* (d4-150, 25 °C).

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## Synthesis of ATP coupled with action of membrane protonic pumps at the octane-water interface

ATP SYNTHESIS in mitochondria is one of the most important energy-producing processes in the cell. We have studied the main stage of this process, the coupling of ATP synthetase to the action of membrane protonic pumps at an octane-water interface.

Two hypotheses exist concerning this coupling of respiration to phosphorylation. That of Williams<sup>1</sup> suggests direct proton transfer in lipid phase from respiration enzymes to ATP synthetase, whereas that of Mitchell<sup>2</sup> postulates an essential additional stage of transmembrane proton transfer, resulting in the formation of a membrane potential. We have carried out ATP synthesis in non-equilibrium conditions (acidic concentration in the hydrophobic phase), thus supporting William's hypothesis.

We had previously shown that the mitochondrial ATPase can transfer protons from water to octane by ATP hydrolysis<sup>3</sup>. We therefore carried out the reverse reaction, and observed ATP synthesis coupled to the action of purified mitochondrial ATPase adsorbed at an octane-water interface. The proton flow through the ATPase complex from octane to water was caused by an excess concentration (with respect to that of the equilibrium) of the undissociated acid or Lewis form in the octane phase.

The excess concentration of acid was produced by oxidation of the reduced form of nicotinamide adenine dinucleotide (NADH) by ferricyanide with the participation of the enzymes comprising the initial step in the respiratory chain of mitochondria. For this purpose, submitochondrial particles (SMP) (for isolation techniques, see refs 4 and 6), were introduced into the octane-water system, to which was added one of the ATPases to be examined and rotenone, an inhibitor of NADH oxidase which prevents respiration and thus the synthesis of ATP in SMP. If NADH is added to the octane-water system containing a proton acceptor 2,4-dinitrophenol (DNP),  $K_3Fe(CN)_6$ , SMP and rotenone, the maximum shift in the positive direction of the potential difference measured in the chain, Au-air-octane, proton acceptor ( $RO^-$ ,  $ROH$ )-water, enzyme, substrate-water, saturated  $KCl-Hg_2Cl_2/Hg$ , was 0.52 V. ATP synthesis at the interface was detected by a decrease in potential difference measured in this chain on addition of ADP and inorganic phosphate to the system. Equilibrium octane-water systems containing 50 mM Tris-HCl (1 mM) and DNP were used.

Enzyme and substrates were added just before measuring the potential differences by the dynamic capacitance method<sup>3,8-10</sup>. Voltages plotted in the figures are referred to the initial value.

When 0.2 mM NADH was added to the system containing SMP,  $K_3Fe(CN)_6$  and oligomycin-sensitive ATPase, a potential shift in the positive direction was observed at the octane-water interface. Subsequent addition of 1 mM ADP and 1 mM inorganic phosphate caused the potential difference to shift in the negative direction. At sufficiently high ADP concentrations this negative shift was below the initial value (Fig. 1). Addition of oligomycin (points C and C', Fig. 1) eliminated almost completely the action of ADP and phosphate on the potential difference in the oligomycin-sensitive ATPase system.

Instead of SMP, another proton pump, such as bacteriorhodopsin sheets (obtained from *Halobacterium halobium*), was used. As we have shown previously<sup>8</sup>, bacteriorhodopsin sheets are also capable of transferring protons from water to octane by the action of light. Figure 2 shows that when light is switched on, bacteriorhodopsin sheets cause a potential change (0.5 V) at the octane-water interface. When the ATPase reaction starts, however, the potential returns to its initial value. When oligomycin is added in the presence of oligomycin-sensitive ATPase of *p-N,N*-di(2-chloroethyl)aminophenylacetic acid, an alkylating agent and inhibitor of soluble mitochondrial ATPase, bacteriorhodopsin sheets again generate the photopotential. Figure 2 shows the change in potential difference in the above chain in the presence of bacteriorhodopsin sheets on illumination and in the dark, as affected by the concentration of

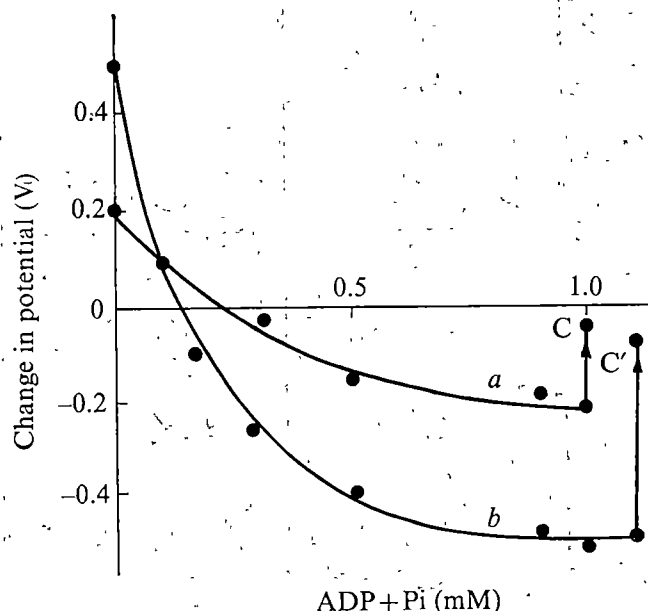


Fig. 1 Shift in negative direction of potential difference in the chain (see text) as affected by the concentration of substrate ADP+Pi. Composition of incubation medium: a 0.1 mg ml<sup>-1</sup> oligomycin-sensitive ATPase, 0.1 mg ml<sup>-1</sup> SMP (isolated by the technique used in ref. 4), 2.5 µg ml<sup>-1</sup> rotenone, 50 mM Tris-HCl, 1 mM DNP, 1 mM MgSO<sub>4</sub>, 1 mM  $K_3Fe(CN)_6$  and 0.2 mM NADH. b, As a except SMP was isolated by the technique used in ref. 2. At points C and C' 2 µg ml<sup>-1</sup> oligomycin was added. The following experiments were run as controls: (1) Oligomycin-sensitive ATPase was excluded from the complete system. In these conditions, addition of ADP and Pi does not affect the potential difference. (2) SMP was excluded from the complete system. Addition of ADP and inorganic phosphate does not affect the potential difference. (3) NADH is excluded from the complete system. A very small potential change (no more than 25 mV) was observed on addition of 1 mM ADP and 1 mM Pi. Inorganic phosphate was excluded from the complete system. A very small potential change (no more than 25 mV) was observed on addition of 1 mM ADP.

Table 1 ATP synthesis at the octane-water interface due to the excess concentration of PCP in octane

ATPase	Experimental conditions	Amount of ATP in sample ( $\times 10^{-8}$ M)	Mean amount of ATP synthesised ( $\times 10^{-8}$ M)
Oligomycin-sensitive ATPase (100 $\mu\text{g ml}^{-1}$ ) + ADP ( $10^{-4}$ M)	-PCB	3.1	—
	+PCB	4.2	—
	+PCB + oligomycin (1 g $\text{ml}^{-1}$ )	2.9	—
	-PCB	2.5	—
Soluble mitochondrial ATPase before heat treatment (130 $\mu\text{g ml}^{-1}$ ) + ADP ( $3 \cdot 10^{-5}$ M)	+PCB	3.7	1.1

the substrate ADP and inorganic phosphate. When ADP and inorganic phosphate are added to a system with an active proton pump, the potential rapidly decreases.

ATP synthesis by oligomycin-sensitive ATPase, adsorbed on the octane-water interface, also takes place after addition of pentachlorophenol-octane solution to the octane phase. The system contained 1 ml octane and 2 ml 20 mM phosphate buffer ( $\text{pH}=7.5$ ) to which were added 40 g hexokinase protein,  $10^{-4}$  M ADP, 20 mM glucose, 2 mM  $\text{MgSO}_4$  and 100  $\mu\text{g}$  protein of oligomycin-sensitive ATPase isolated from the mitochondria of ox heart by Racker's method<sup>2</sup>. After introduction of 0.02 ml PCP into octane, its concentration in the complete system was  $5 \times 10^{-5}$  M. After 1 min. the reaction was stopped by perchloric acid and neutralised with NaOH. The amount of ATP formed was determined fluorimetrically by the glucose-6-phosphate dehydrogenase method. In the control tests PCP and octane were excluded from the system. The results of these tests (Table 1) show that in the control tests ATP is present as an impurity in the reactants used and can be formed according to the equation  $2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$ , because of the presence of myokinases in the enzymes used. In the complete system the amount of ATP formed was 30–50% higher than in control tests in which PCP was not added. The experimental error was no greater than 10%. In the experiments with oligomycin-sensitive ATPase, oligomycin lowered the ATP level to the control value.

Figure 3 shows a scheme for the action of ATPase at the interface, based on the experimental data described above. It is clear from this scheme that under the action of light, in the presence in the non-aqueous phase of a proton acceptor (such as a weak acid, ROH), bacteriorhodopsin transfers protons to this phase. Of course, protons do not exist in the free form in the non-aqueous phase, but are

captured by  $\text{RO}^-$  or ROH to form ROH or  $\text{ROH}_2^+$  (Lewis acid form<sup>10</sup>).

If the charged Lewis acid form alone acted in this process as proton donor, the potential would always be positive and equal to, or less than, the initial positive charge of octane obtained in the case of SMP (Fig. 1). The change in the potential sign in the negative direction observed in the experiment with SMP must mean that ATP synthesis is also brought about by the elimination of protons from the neutral ROH form of DNP. In this case the anion remains in the non-aqueous phase and the proton goes into water. To verify this assumption, we used dimethoxybenzene

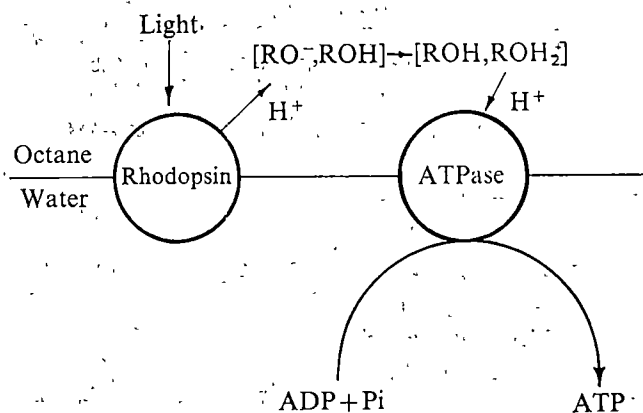
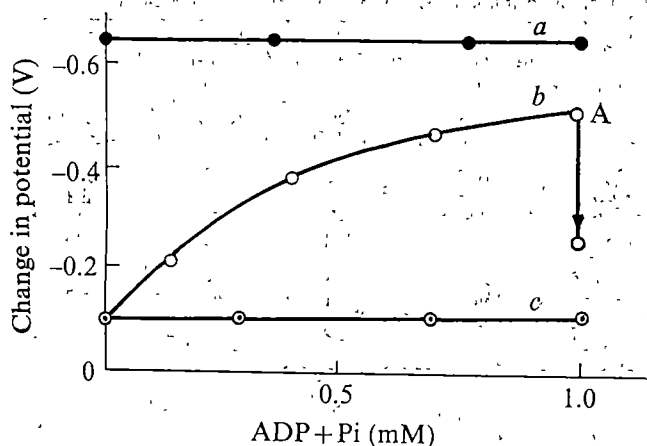


Fig. 3 Scheme for ATP synthesis at octane-water interface brought about by action of bacteriorhodopsin sheets.

Fig. 2 Dependence of potential difference measured in the chain (see text) on the concentration of substrate ADP + Pi. Composition of incubation medium: a, 30  $\mu\text{g ml}^{-1}$  bacteriorhodopsin sheets, 1 mM DNP, 50 mM Tris-HCl, and 1 mM  $\text{MgSO}_4$  in the dark; b, as c plus  $10^{-8}$  M soluble mitochondrial ATPase; c, as a, b and c under illumination.  $\text{pH} = 7.9$ . At A, 1 mM *p*-N,N-di-(2-chloroethyl)aminophenylacetic acid has been introduced into the cell



instead of DNP as Lewis-type proton acceptor in the non-aqueous phase. In this case, when NADH is added to the system containing soluble mitochondrial ATPase and SMP, the potential shifts in the positive direction by 0.2 V. At high ADP and inorganic phosphate concentrations it does not shift in the negative direction.

The interpretation of our experimental data obtained from measurements of the potential difference in the above chain is being verified by direct determinations of the amount of ATP synthesised. Our data show that the presence of a transmembrane potential is not a necessary condition for ATP synthesis by ATPase. The energy of proton (acid) solvation can be used for ATP synthesis.

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## Antiviral, immunosuppressive and antitumour effects of Ribavirin

THE compound 1- $\beta$ -D-ribofuranosyl-2,2,4-triazole-3-carboxamide (Ribavirin) has been reported to inhibit the replication of both RNA and DNA viruses *in vitro*, and to inhibit influenza virus infection of tissue cultures and mice<sup>1-3</sup>. The compound acts by interfering with guanine monophosphate formation and subsequent nucleic acid synthesis; at a concentration which completely inhibited influenza viral polypeptide production in tissue culture, however, there was no demonstrable effect on cellular protein synthesis<sup>4</sup>. To test the activity of Ribavirin further, we examined the effect of this compound on influenza virus

**Table 1** Response of ferrets to infection with influenza virus A/Port Chalmers/73

	Control ferrets	Ferrets treated with Ribavirin (100 mg kg <sup>-1</sup> d <sup>-1</sup> )
Response to infection*		
Rise in temperature† (°C)	39.3-40.9	39.6-39.6
Virus titre in nasal wash at 48 h post-infection (EID <sub>50</sub> ml <sup>-1</sup> )	10 <sup>6.30</sup>	10 <sup>4.35</sup>
Nasal wash protein concentration, pre- and 7 d post-infection (mg per 100 ml)	0.28-1.22	0.26-0.43
Nasal wash neutralising antibody titre; pre- and 7 d post-infection	<2-1:14	<2-<2
Serum HI antibody response (g.m.t.)	<5-463	<5-<5

\*Mean values for four ferrets.

†Increase in temperature from mean, pre-inoculation temperature to temperature at 48 h post-infection.

infection of ferrets. Four adult ferrets were inoculated intraperitoneally with 100 mg kg<sup>-1</sup> d<sup>-1</sup> of Ribavirin each for 7 d, this dose although it did not cause deaths or other signs of toxicity, was probably close to the toxic level as judged by standard tests in mice. Two hours after the second inoculation of drug, these animals and four

untreated ferrets were lightly anaesthetised and inoculated intranasally with 10<sup>3.0</sup> ferret-infective doses of egg-grown influenza virus A/Port Chalmers/73 (H3N2). Following virus infection, the drug-treated ferrets were given 5 further doses of Ribavirin. The response of the animals to virus infection was measured, as described previously<sup>5,6</sup>. The results are shown in Table 1. Ribavirin had a marked effect on the response of ferrets to influenza virus infection; compared with control ferrets, influenza virus-infected animals treated with Ribavirin did not exhibit a febrile reaction to infection, did not develop a significant increase in nasal wash protein and did not develop either local or serum antibody. In addition, the titre of virus in nasal washings collected 3 d after virus infection from drug-treated animals was 100-fold less than that found in control ferrets.

The absence of both a serum and a local antibody response in Ribavirin-treated ferrets infected with influenza virus may have been due to the antiviral activity of the compound which reduced virus replication to a level that did not induce antibody production (Table 1). Alternatively, Ribavirin may also have an immunosuppressive effect. To test this latter possibility, a group of guinea pigs was inoculated intraperitoneally with 100 mg kg<sup>-1</sup> d<sup>-1</sup> of Ribavirin for 10 d. On the second day, the drug-treated animals and a group of untreated guinea pigs were inoculated intramuscularly with 400 IU of inactivated influenza virus A/England/42/72 (H3N2) vaccine in an equal volume of Freund's complete adjuvant (FCA). Serum samples were collected before and 10 d after immunisation, and tested for serum haemagglutination inhibiting (HI) antibody. Some guinea pigs in each group were inoculated intraperitoneally with 10 ml of sterile paraffin 4 d after immunisation and were killed 6 d later; the peritoneal macrophages were collected from these animals and tested for cell-mediated immunity to influenza virus by the macrophage migration inhibition test. Ten days after immunisation, peripheral blood specimens were collected from the guinea pigs, and the buffy coat cells incubated with 2.0  $\mu$ g ml<sup>-1</sup> of phytohaemagglutinin and tested for lymphocyte transformation. The results are shown in Table 2. Guinea pigs immunised with influenza virus vaccine in FCA produced serum HI antibody by 10 d, post-inoculation (geometric mean titre g.m.t.=1:124); at this time however, no antibody was detectable in serum from Ribavirin-treated animals. The migration of peritoneal macrophages from both control and drug-treated guinea pigs was significantly inhibited in the presence of 40 IU of influenza virus A/England/42/72 vaccine. Furthermore, lymphocytes from drug-treated and control animals were both transformed by PHA to approximately the same degree (Table 2). The results indicate that Ribavirin, at

**Table 2** Immune response of guinea pigs to inoculation with 400 IU of inactivated influenza virus A/England/42/72 vaccine in FCA

Ribavirin treatment	Animal no.	Serum HI antibody response (g.m.t.)		Delayed hypersensitivity response (MMI test) to 40 IU influenza vaccine at day 10, post-infection			Response to PHA (2.0 $\mu$ g ml <sup>-1</sup> ) at day 10, post-inoculation		
		day -1	day 10	Test $\pm$ s.d.	Control $\pm$ s.d.	Inhibition %	Test (c.p.m. $\times 10^{-4}$ )	Control (c.p.m. $\times 10^{-4}$ )	Fold stimulation
100 mg kg <sup>-1</sup> d <sup>-1</sup> from day -1 to 10	1	<5	<5	NT	NT	NT	22.2	2.1	10.7
	2	<5	<5	0.25 $\pm$ 0.01	0.48 $\pm$ 0.04	54†	7.9	1.3	6.1
	3	<5	<5	0.66 $\pm$ 0.02	1.25 $\pm$ 0.11	47†	NT	NT	NT
	4	<5	<5	NT	NT	NT	NT	NT	NT
Nil	5	<5	512	0.75 $\pm$ 0.05	1.79 $\pm$ 0.22	58†	NT	NT	NT
	6	<5	128	1.33 $\pm$ 0.68	2.91 $\pm$ 0.57	54†	NT	NT	NT
	7	<5	32	0.47 $\pm$ 0.06	0.73 $\pm$ 0.02	36†	13.2	1.7	7.8
	8	<5	128	0.31 $\pm$ 0.01	0.40 $\pm$ 0.01	22†	6.3	1.7	3.7

\*Mean area of macrophage migration  $\pm$  standard deviation.

†Significant inhibition of macrophage migration ( $P = < 0.05$ ).

NT, not tested.

Table 3 Effect of Ribavirin on transplanted adenovirus 12-induced tumours of CBA mice

Experiment no.	Ribavirin treatment	Incidence of tumours, days post-inoculation (mean tumour diameter in cm)					
		7	14	17	21	24	26
1	100 mg kg <sup>-1</sup> daily from day -1 to day 21	0/8	0/8	1/8 (0.2)*	2/8 (0.2)	4/8 (0.4)	4/8 (0.7)
	Nil	0/8	2/8 (0.3)	4/8 (0.3)	5/8 (0.7)	6/8 (1.0)	7/8 (1.5)
2	100 mg kg <sup>-1</sup> daily from day -1 to day 21	0/8	0/8	0/8	2/8 (0.1)	5/8 (0.3)	5/8 (0.5)
	Nil	0/8	2/8 (0.1)	4/8 (0.3)	5/8 (0.5)	8/8 (1.0)	8/8 (1.4)

\*No. of mice with tumours/no. of mice inoculated (mean tumour diameter in cm)

the concentration and by the route of inoculation used, inhibited the serum antibody response to influenza virus vaccine, but did not inhibit the cellular immune response.

Previous studies have shown that several compounds, such as ethidium bromide, rifamycin and Tilorone inhibit the RNA-dependent DNA polymerase of oncornaviruses and have an antitumour activity<sup>7-10</sup>; indeed, if oncornaviruses are important in maintaining the cancerous properties of transformed cells, the two properties may be related. Thus, Tilorone has a chemotherapeutic action against a wide range of viruses, and is an interferon inducer<sup>11</sup>; however, the antitumour activity of different analogues of this compound correlated more closely with the inhibitory activity on viral DNA polymerase, than with the ability to induce interferon<sup>12</sup>. From these observations, it is possible that many antiviral compounds, such as Ribavirin, may have antitumour activity. To test this possibility, groups of CBA mice were inoculated subcutaneously with 100 mg kg<sup>-1</sup> d<sup>-1</sup> of Ribavirin. Two hours after the second drug inoculation, these animals, together with a group of untreated mice, were inoculated subcutaneously at a different site with 5 × 10<sup>5</sup> transplanted adenovirus 12-induced CBA mouse tumour cells; the tumour cell inoculum used represented ten (50%) tumour doses<sup>13</sup>. Ribavirin was given to the treated mice for a further 20 d, and the incidence and size of tumours in the animals was recorded twice weekly for 4 weeks. Two identical experiments were carried out, and the results are shown in Table 3. In both experiments, the incidence of tumours and the size of palpable tumours was greater in control mice than in Ribavirin-treated mice; thus, Ribavirin inhibited the growth of transplanted adenovirus 12 tumours.

It is not certain from these studies that the antitumour activity of Ribavirin was due to the action of the drug on oncornaviruses present in the tumour cells; there are alternative mechanisms. The immune response of animals and man to either experimental or natural tumours has been shown to be cell mediated<sup>14,15</sup>, and factors which promote the cellular immunity of the host may enhance tumour immunity<sup>16</sup>. In contrast, sera from tumour-bearing animals may contain blocking factors which interfere with the cell-mediated immune response, and the production of humoral antibody could enhance tumour growth<sup>17</sup>. By allowing a cell-mediated response to transplanted tumour cells and inhibiting the humoral antibody response, as shown in the present studies in guinea pigs, the antitumour activity of Ribavirin may be due to the effect of this compound on the immune response of the host, and an enhancement of the mechanism of tumour cell rejection. By which mechanism or combination of mechanisms Ribavirin exerts an antitumour activity is not known; however, it is possible that the effectiveness of the compound is a result of a direct action on the oncornavirus present in tumour cells. If this is so, other antiviral compounds may share this property, and this should be investigated.

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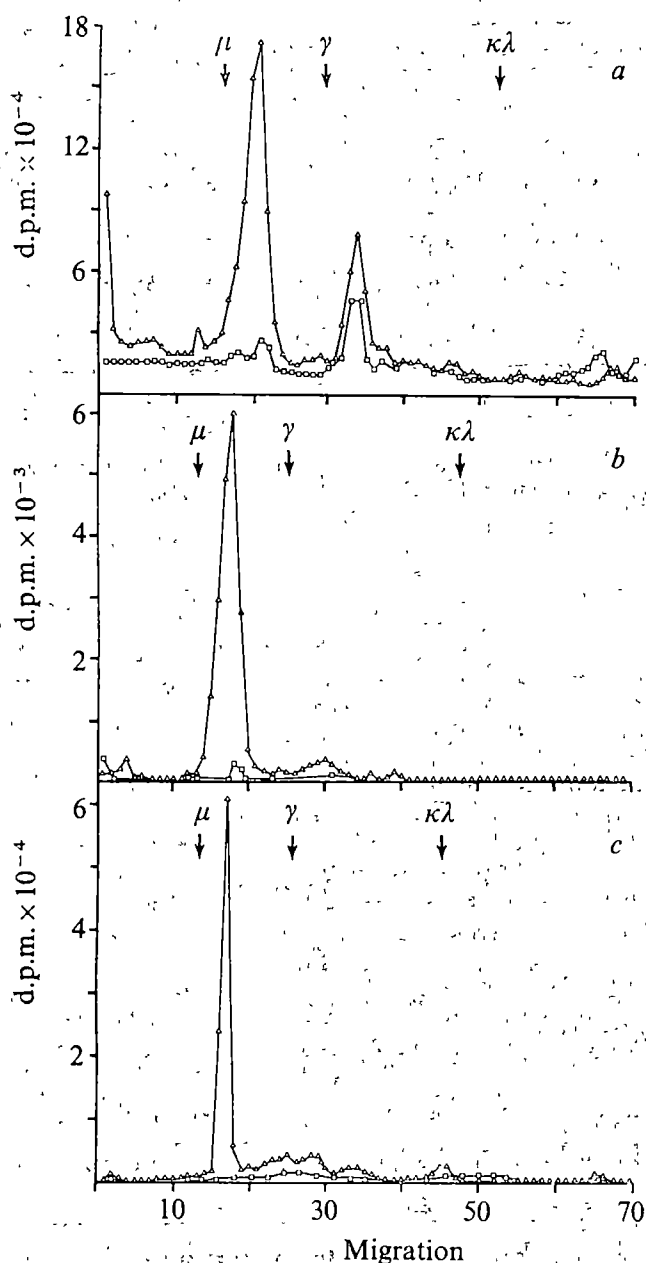
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## Relationship between the oncornavirus gene product gp70 and a major protein secretion of the mouse genital tract

SEVERAL investigators have postulated that endogenous oncornaviruses play a role in development and differentiation, and that neoplasia is an unfortunate consequence of an otherwise important symbiosis<sup>1,2</sup>. Although attractive on theoretical grounds, these concepts remain unproven. Nevertheless, it is possible to study the relationship between viral gene expression and normal host functions. Evidence from several laboratories has suggested that expression of endogenous oncornavirus genes are under differentiation control in the mouse<sup>3-6</sup>. The clearest example of this is the case of gp70, the major envelope glycoprotein of the murine leukaemia viruses (MuLV)<sup>7-11</sup>. This protein which is coded for by the viral genome<sup>12</sup> may be a component of the surfaces of cells which follow certain pathways of differentiation. Further, the thymocyte differentiation marker, G<sub>1x</sub>, has been shown to be a type-specific antigenic determinant of some gp70 molecules<sup>4,5</sup>.

Although, as studies of G<sub>1x</sub> suggested, expression of gp70 is linked to cellular differentiation, we have shown already that its control is more relaxed than previously recognised. By immunofluorescence, gp70 was detected in various cell types, particularly lymphoid and epithelial



**Fig. 1** Characterisation of MuLV gp70 in secretions of epididymis and ductus deferens. Secretions of the epididymis and ductus deferens of an NZB mouse and disrupted MuLV (Scripps) were radioiodinated by the chloramine T method<sup>11</sup>. MuLV-producing lymphoblastoid cells (SCRF 60 A) were surface radioiodinated using lactoperoxidase as described previously<sup>8,12</sup>. After washing, cells were disrupted with 0.5% Nonidet P-40 and sonication, then centrifuged at 100,000g for 1 h. The resulting supernatant was used for immune precipitation. Labelled preparations were analysed by indirect immune precipitation as described in detail before<sup>8,9</sup>. Either goat anti-gp70 (Scripps) or normal goat serum was used as the primary antibody and rabbit anti-goat IgG was the second antibody. Resulting immune precipitates were dissociated in 8-M urea, 1% SDS, 2%  $\beta$ -mercaptoethanol, and then analysed on 6  $\times$  100 mm SDS polyacrylamide using the Laemmli system<sup>16</sup>. <sup>125</sup>I-radioiodinated  $\mu$ ,  $\gamma$ , and  $\kappa\lambda$  marker proteins from human IgG were included in each gel. Gels were sliced and counted as described before<sup>8</sup>. Results were corrected for crossover and counting efficiency and were plotted with a Hewlett-Packard system. Position of the marker protein is indicated by arrows. *a*, Radioiodinated SLV proteins reacted with goat anti-gp70 ( $\Delta$ ) or with normal goat serum ( $\square$ ). This figure shows that the goat anti-gp70 reacts primarily with MuLV gp70. The gp45 in this gel is a breakdown product of gp70, a subject which is now being studied. *b*, Radioiodinated SCRF 60 A cell surface proteins reacted with goat anti-gp70 ( $\Delta$ ) or with normal goat serum ( $\square$ ). Again, the specificity of the goat anti-gp70 for MuLV gp70 is shown. *c*, Radioiodinated secretions from the genital tract of a NZB mouse reacted with goat anti-gp70 ( $\Delta$ ) or with normal goat serum ( $\square$ ). This figure demonstrates that gp70 is a component of the secretions of the male genital tract. The approximate molecular weight of the gp70 from each source is 67,000.

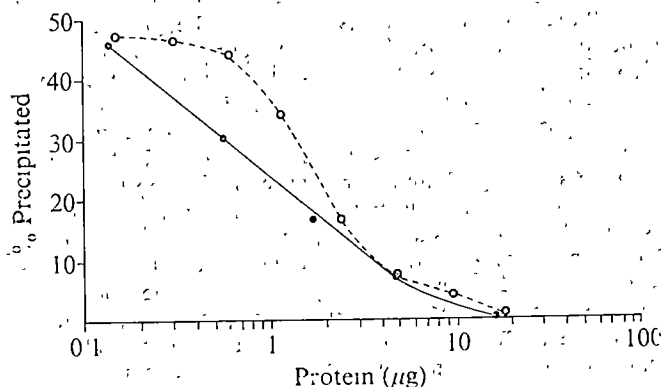
cells<sup>13</sup>. We now report that a protein related immunologically to gp70 is present in the secretions of the epididymis and ductus deferens of the mouse. This protein has a molecular weight of 67,000, comprises up to 10% of these secretions, and is associated with the surface of sperm. Apart from its biological importance, identification of a major site of expression of an oncornavirus-related protein allows us to complement serological analysis with a structural study in which a virion structural protein is compared with a differentiation antigen of the mouse.

The molecular properties of the gp70 molecules of MuLV and of the secretions of the epididymis and ductus deferens were compared by sodium dodecyl-sulphate (SDS) polyacrylamide gel electrophoresis of indirect immune precipitates and by analysis of peptide maps of proteins eluted from gels. The data in Fig. 1 show that the anti-gp70 antibody reacts specifically with a single component from each of three sources: (1) purified MuLV (Scripps), (2) surface radioiodinated MuLV-producing lymphoblastoid cells (SCRF 60 A) or (3) secretions from the epididymis and ductus deferens. The molecular weight of the protein was approximately 67,000 in all three cases. A comparison of the tryptic peptides of AKR virus gp70 and genital tract protein from AKR mice showed significant differences (B.C.D.V. *et al.*, in preparation). We do not yet know if the observed differences are due to glycosylation or change in the amino acid composition.

Antigenic cross reactivity between MuLV gp70 and the gp70 of the genital tract was confirmed by double diffusion studies. Antibodies against purified MuLV gp70 gave a reaction of partial identity between MuLV gp70 and a molecule in the secretions of epididymis and ductus deferens. MuLV gp70 has antigenic determinants not present on the genital tract protein, resulting in a 'spur' in the Ouchterlony plate. After absorption of anti-gp70 antibody with a crude preparation of secretions from the epididymis and ductus deferens, the reaction between the absorbed antiserum and the genital tract protein was abolished whereas the reaction between the absorbed antiserum and the viral gp70 was not. Conversely, we showed that absorption of the anti-gp70 with virus abolished reaction to both viral and genital tract protein.

The amount of gp70 in preparations of the secretions from the genital tract was determined by competition radioimmunoassay using the interspecies assay. As discussed by Strand and August<sup>9</sup>, such competition curves are the result of three parameters of the reaction: first, the concentration of the competing proteins; second, the relative affinity of the antibodies for the proteins; and, third, the composition of the antigenic determinants of the proteins and of the

**Fig. 2** Competition radioimmunoassay for MuLV gp70. Reaction conditions were similar to those of Strand and August<sup>9</sup> in which the interspecies determinants of gp70 are measured, goat anti-FeLV (1:700) and <sup>125</sup>I-gp70 (Friend) (1 ng). Competing proteins were from MuLV (Scripps) ( $\circ$ ) and from secretions of epididymis and ductus deferens of an NZB mouse ( $\bullet$ ). Similar curves for the genital tract gp70 were obtained for NZB (NZB  $\times$  NZW)F<sub>1</sub>, NZW and 129 G<sub>1</sub>X<sup>+</sup>.



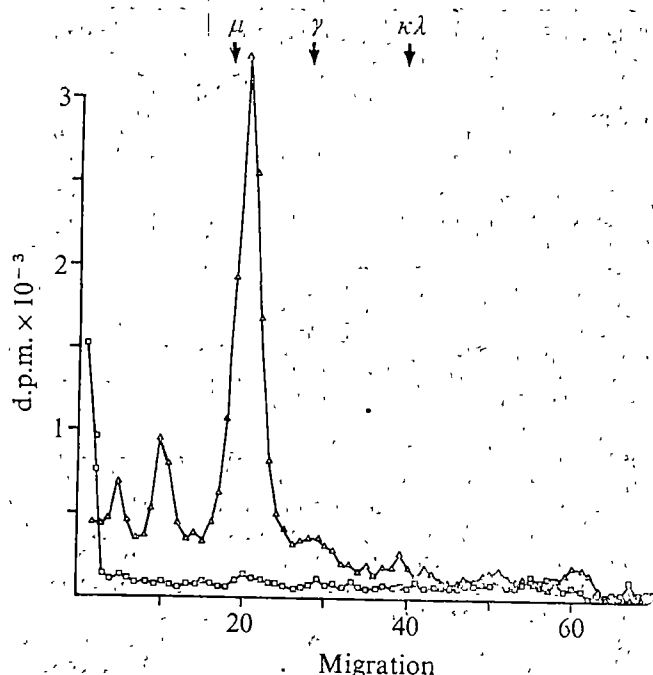


Fig. 3 Association of MuLV gp70 with sperm. NZB sperm were purified from epididymis and ductus deferens by differential centrifugation. Sperm were surface radioiodinated with lactoperoxidase, then extracted and analysed. SDS polyacrylamide gel electrophoresis of radioiodinated sperm proteins reacted with goat anti-gp70 (Scripps) ( $\Delta$ ) or with normal goat serum ( $\square$ ). Position of  $^{131}\text{I}$  marker proteins is indicated by arrows. The approximate molecular weight of the sperm gp70 is 67,000.

antibodies. A set of representative binding curves is shown in Fig. 2. The relative amount of gp70 in the secretions of the genital tract varied considerably from preparation to preparation, ranging from 2 to 10% of the protein. This variation may reflect different amounts of gp70 in the secretions from mouse to mouse, and from strain to strain, but was also undoubtedly influenced by the extent to which the secretions were recovered and/or contaminated by other proteins, especially serum proteins.

The relative slope of the inhibition curves for MuLV gp70 and that of the genital tract proteins are different, indicating differences in the affinities of the interspecies antigenic determinants for the antibodies. At high concentrations the genital tract proteins compete completely for antibody with the labelled viral gp70. Thus, most of the viral interspecies determinants are present in the genital tract protein. These studies show that the MuLV gp70 and the molecules from the genital tract are immunologically similar, but not identical.

Proteins of epididymal secretions are known to associate with the surface of sperm where they may play a role in capacitation. To determine whether gp70 associated with the sperm surface, epididymal sperm were purified and then surface radioiodinated. Figure 3 shows that in this strain, a gp70 molecule is associated with the sperm surface. It is interesting that studies (to be reported elsewhere) have shown that the gp70 of the epididymal secretions and that associated with the sperm surface may be the product of different genes.

The basic question relating to the proteins studied here is whether the host and viral proteins are transcripts from a single gene and are modified after synthesis as by glycosylation, or whether they are products of different genes. The differences in peptide maps strongly suggest that they are different gene products. A possibility not excluded by our studies is that MuLV gp70 and the genital tract protein are unrelated gene products modified so as to cross react immunologically. But a trivial cross reactivity seems un-

likely for several reasons. First, three different antisera prepared against purified gp70 from three different FMR viruses all reacted with the genital tract protein. Second, we know from previous studies that at least one protein of the male genital tract shows a mode of inheritance identical to that of  $G_{IX}$  and is lost in congenic  $G_{IX}^-$  mice<sup>13</sup>. Third, the interspecies determinants of viral gp70 are completely represented in the genital tract protein. Finally, the cross reactivity between viral and genital tract gp70 is not simply due to common carbohydrate groups. Unpublished results from our group and Bolognesi's group (personal communication) show that proteolysis destroys the antigenicity of gp70 whereas removal of the carbohydrate does not.

The data presented here indicate that several distinct genes for gp70 can be found in the mouse genome, some of which code for proteins made by certain differentiated cells; and others which code for proteins which are incorporated into virus.

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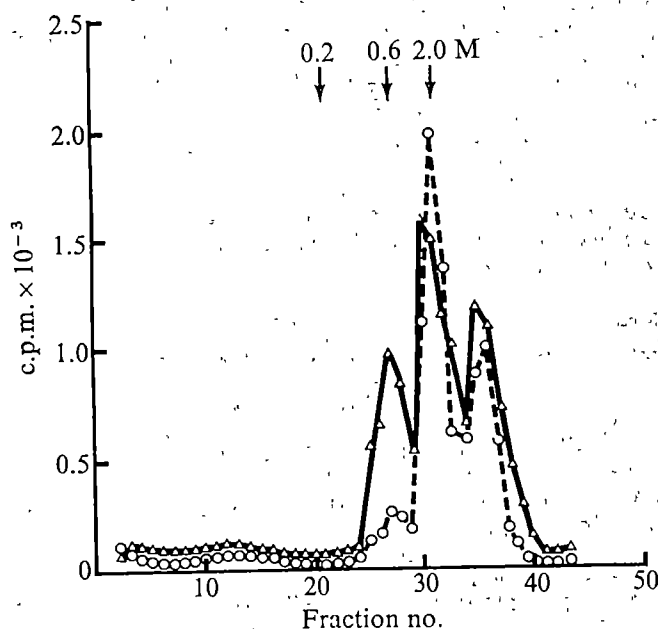
## Isolation of nucleic acid-binding protein: stimulation of reverse transcriptase-catalysed DNA synthesis

RNA tumour viruses transfer their genetic information from a single-stranded RNA genome found in their virions to a double-stranded DNA covalently integrated into chromosomes of the infected host<sup>1,2</sup>. A series of nucleic acid intermediates must therefore exist between the single-stranded RNA and the integrated viral DNA genome. The discovery of RNA-directed DNA polymerase (reverse transcriptase) associated with RNA tumour viruses fulfilled a critical requirement for synthesis of viral DNA<sup>3,4</sup>. The initial product in this information transfer is a RNA-DNA



hybrid which is converted into free, unintegrated DNA-DNA<sup>8</sup>. The double-stranded DNA (provirus) is then integrated into chromosomes of the infected host. We report here the isolation of a nucleic acid-binding (unwinding) protein from chick fibroblasts transformed by Rous sarcoma virus (RSV) and its stimulatory effect on DNA synthesis catalysed by reverse transcriptase. A protein capable of unwinding RNA-DNA hybrid and DNA-DNA duplex would not only conserve the input viral RNA strand for further reverse transcription, but also facilitate replication of the double-stranded viral DNA into many copies of provirus before integration.

Binding protein was isolated by affinity chromatography from chick embryo fibroblasts chronically transformed by Schmidt-Ruppin RSV, subgroup D after 2 weeks of virus infection. Cellulose containing covalently linked single-stranded calf thymus DNA was purchased from P.L. Biochemicals. All radioactive polynucleotides were purchased from Miles Laboratories. In a typical run, about 2 wet g transformed cells were washed, sonicated and centrifuged to obtain debris-free supernatant. The supernatant was treated with polyethylene glycol and centrifuged to remove nucleic acid. After dialysis the supernatant was



**Fig. 1** Isolation of DNA-binding protein using DNA-cellulose column. Frozen chicken embryonic fibroblasts (2 g), completely transformed by RSV, subgroup D, were suspended and washed twice with phosphate-buffered saline. Cells suspended in 4 ml sonicating buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 1.7 M NaCl) and disintegrated for 45 s at 4 A in a Branson Sonifier. Cell debris removed by centrifugation at 10,000g for 10 min. Polyethylene glycol (MW 6,000–7,500, 30% in 1.7 M NaCl) was stirred into supernatant to a concentration of 10%. After stirring for 30 min, suspension centrifuged at 27,000g to remove nucleic acid precipitate. Resulting clear supernatant fraction dialysed against 2 l Tris buffer (20 mM, Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 50 mM NaCl, 40% glycerol) for 20 h. Precipitate formed during dialysis removed by centrifugation (27,000g for 20 min) and supernatant passed through (4 ml h<sup>-1</sup>) a column (1 × 1 cm) of single-stranded DNA-cellulose (P.L. Biochemicals) that had been washed with the Tris buffer. Column washed with 10 ml Tris buffer and eluted with 2.5 ml each of 0.2 M, 0.6 M, 2.0 M NaCl in Tris buffer. Fractions (0.5 ml) were collected. <sup>3</sup>H-poly d(AT)-poly d(AT) (2 nmol, 25 mCi mmol<sup>-1</sup> P) or <sup>3</sup>H-poly(rA)-poly(dT) (2.8 nmol, 18 mCi mmol<sup>-1</sup> P) incubated with fractions (10 µl) in binding buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 2 mM mercaptoethanol, 50 mM NaCl and 5% glycerol) in total volume 0.1 ml at 35 °C for 10 min. Solution chilled and diluted with 2 ml binding buffer and filtered through Millipore filter. Filter washed 3 times with 3 ml binding buffer; dried and radioactivity determined in toluene-based scintillation fluid.  $\Delta$ , Binding of <sup>3</sup>H-poly d(AT)-poly d(AT);  $\circ$ , binding of <sup>3</sup>H-poly(rA)-poly(dT).

**Table 1** Effect of binding protein (BP) on DNA synthesis by reverse transcriptase

Template	pmol <sup>3</sup> H-dTMP incorporated per h*		Ratio +BP
	–BP	+BP	–BP
Native calf thymus DNA	0.86	1.50	1.7
Nicked calf thymus DNA†	0.88	2.62	3.0
Heated calf thymus DNA‡	0.80	11.0	13.7

\*Reaction conditions as in Fig. 2 except 0.9 U reverse transcriptase were used. Binding protein, when present, was 0.5 µg per incubation. DNA concentrations were 50 µg ml<sup>-1</sup>.

†Calf thymus DNA nicked by treatment with DNase I (ref. 11).

‡Calf thymus DNA was heat-denatured at 100 °C for 15 min and chilled immediately<sup>11</sup>.

passed through a DNA-cellulose column (1 × 1 cm) pre-equilibrated with Tris buffer (20 mM, Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 50 mM NaCl and 10% glycerol). The column was washed with the Tris buffer and then eluted stepwise with 0.2, 0.6 and 2.0 M NaCl in the buffer. Resulting fractions were assayed for nucleic acid binding activity as described previously<sup>7</sup> using either <sup>3</sup>H-poly d(AT)-poly d(AT) or <sup>3</sup>H-poly(rA)-poly(dT) as substrate. Fig. 1 shows the results obtained from the DNA-cellulose column. There were three peaks of binding activity when the radioactive templates were used. All the fractions containing binding activity were combined and dialysed against the binding protein buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM 2-mercaptoethanol, 20 mM NaCl and 30% glycerol).

**Table 2** Effect of binding protein (BP) on reverse transcription of RSV 70S RNA

Template	Binding protein	pmol <sup>3</sup> H-dTMP incorporated*	+BP % –BP
70S†	0	0.65	—
70S	0.8 µg	1.08	1.6
70S	2.0 µg	1.54	2.4
—	2.0 µg	0	—

\*Reaction conditions as in Fig. 2, except 0.5 U purified reverse transcriptase from RSV B77 used.

†RSV 70S RNA (4.5 µg ml<sup>-1</sup>) isolated from RSV B77 by sucrose density centrifugation<sup>24</sup>. 70S peak showed a single band of high molecular weight material in gel electrophoresis<sup>25</sup>.

The effect of the isolated binding protein on polymerase activity was studied in a reaction in which reverse transcriptase<sup>8</sup> catalysed DNA synthesis using heat-denatured calf thymus DNA as template. DNA synthesis as represented by TCA-insoluble counts<sup>9</sup> was linearly proportional to the amount of binding protein<sup>10</sup> present in the reaction with a maximum stimulation of about tenfold increase with 1 µg of the protein (Fig. 2). Heating of the binding protein at 100 °C for 5 min completely abolished the stimulatory effect and the binding protein itself contained no polymerase activity (Fig. 2).

It is clear from Figs 1 and 2 that the isolated protein binds to DNA and stimulates reverse transcriptase in DNA synthesis. We explored the possibility whether the binding protein enhances DNA synthesis by promoting denaturation (unwinding) of double-stranded DNA. When native calf thymus DNA was used as template the binding protein stimulated DNA synthesis by reverse transcriptase about twofold, which is not inconsistent with the unwinding of the template (Table 1). When the template was native DNA nicked slightly with DNase<sup>11</sup>, the protein stimulated DNA synthesis even greater (threefold), suggesting easier unwinding of the double-stranded DNA after nicking. When heat-denatured DNA was used as template, denatured DNA in the absence of the protein was a poor template for

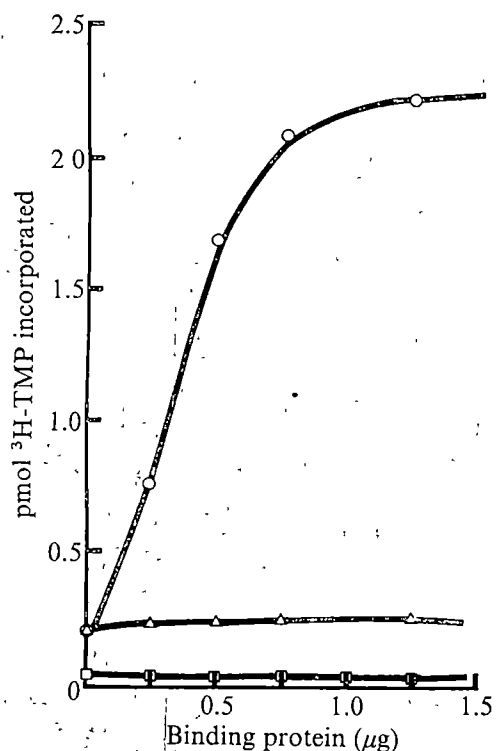


Fig. 2 Stimulation of reverse transcriptase DNA synthesis by binding protein. Reaction mixture in total volume of 120  $\mu$ l contained: purified reverse transcriptase<sup>8</sup> (phosphocellulose fractions, eluted with 0.22 M phosphate buffer), 0.3 U; dATP, dGTP, dCTP, 0.1 mM each; MgCl 5 mM; dithiothreitol, 3 mM; NaCl, 50 mM; heat-denatured calf thymus DNA, 6  $\mu$ g (Sigma, type 1; boiled at 100 °C for 15 min and chilled); <sup>3</sup>H-dTTP, 8  $\mu$ Ci (20  $\mu$ Ci nmol<sup>-1</sup>); and specified amount of binding protein as determined by Lowry *et al.*<sup>10</sup>. Incubation carried out at 35 °C for 60 min and TCA-insoluble radioactivity determined by filter paper method. One unit of reverse transcriptase activity was defined as ability to incorporate 1 pmol <sup>3</sup>H-dTTP into TCA-insoluble precipitate per h in experimental conditions described above. ○, Reverse transcriptase activity in presence of binding protein; Δ, in presence of heated binding protein; □, polymerase activity of binding protein alone.

reverse transcriptase, which is in agreement with the observation that reverse transcriptase did not prefer single-stranded DNA<sup>12</sup>. Binding protein, however, stimulated greatly (14-fold) DNA synthesis when heat-denatured DNA template was used. We suggest that the protein binds strongly and cooperatively to single-stranded DNA and thus promotes unwinding of the small region of undenatured, double-stranded segment to which reverse transcriptase is attached. When bound to single-stranded DNA, it prevents intrastrand annealing and maintains the DNA in an extended conformation, thereby making the template more transcribable.

That the binding protein can unwind double-stranded DNA was studied in a separate experiment. When an excess of the binding protein (78  $\mu$ g) was added at 24 °C to 8  $\mu$ g double-stranded DNA, poly d(AT):poly d(AT),  $A_{260}$  of the DNA increased as a function of time until a plateau 35% greater than the original absorbance was reached (Fig. 3). Similar results were obtained using poly(rA):poly(dT), poly(A):poly(U) and 70S RSV RNA as substrates; the hyperchromic effects were about 29%, 19% and 20%, respectively, in the conditions used (not shown here). This hyperchromic shift is characteristic of the helix-coil transition which has been observed with the binding protein from bacteria<sup>13-15</sup>.

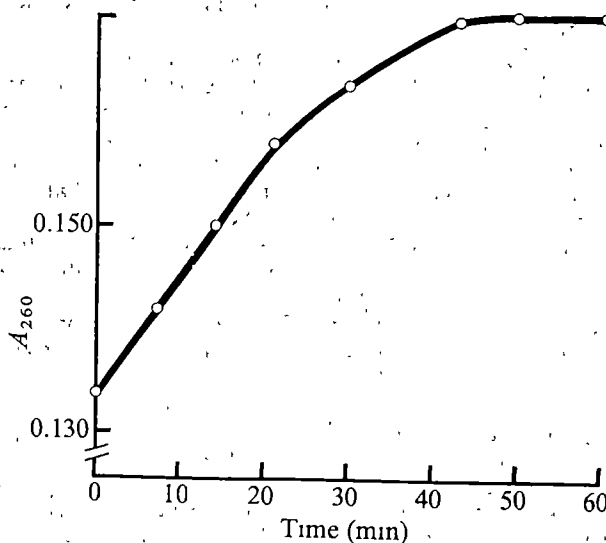
The binding protein reported here was capable of binding to all forms of nucleic acids: double-stranded DNA, <sup>3</sup>H-poly d(AT):poly d(AT), single-stranded DNA (affinity

column), double-stranded RNA (<sup>3</sup>H-poly(A):poly(U)), single-stranded RNA (<sup>3</sup>H-poly(A)) and DNA-RNA hybrid (<sup>3</sup>H-poly(rA):poly(dT)). The binding affinity in preliminary studies indicated about the same preference by the protein for double-stranded DNA or RNA-DNA hybrid. The affinity to single-stranded RNA (<sup>3</sup>H-poly(U)), however, was greater than double-stranded RNA (<sup>3</sup>H-poly(A):poly(U) or <sup>3</sup>H-poly(I):poly(C)).

The protein isolated by the DNA affinity column contained some DNase activity. Details of purification and properties will be described elsewhere. As in the case of the reported specificity of binding proteins to polymerases from the same source<sup>13,15-18</sup>, the binding protein stimulated greatly reverse transcriptase but little, if any, polymerase from *M. lysodeikticus* (data not shown). Although the binding protein stimulated sevenfold DNA synthesis by bacterial polymerase using native DNA as template, it stimulated little, if any, DNA synthesis after the template was heat denatured, suggesting that the stimulation was attributable to unwinding of the template by the protein instead of interaction between the binding protein and the bacterial polymerase. In contrast to this, the binding protein stimulated 14-fold DNA synthesis by reverse transcriptase on the heat-denatured template, suggesting that the binding protein, in addition to unwinding DNA, may also bind to reverse transcriptase during synthesis in such a way that the enzyme does not dissociate from the template and thus prolongs synthesis. We are studying the possibility of such an interaction between reverse transcriptase and the binding protein analogous to that of polymerase II and the binding protein in *Escherichia coli*<sup>13,16</sup>.

We have yet to determine the exact relationship of the binding protein reported here to viral RNA replication. We have also isolated a binding protein from chick embryo fibroblasts similar to that from RSV-transformed cells. Therefore, it seems that the binding protein is not coded by the virus. Intramolecular hydrogen bonding of 70S RSV genome is well known<sup>19</sup>. In the presence of the binding protein in a preliminary study, we have observed in electron microscopy an extended form of RSV RNA instead of the usual, collapsed structure, similar to the observation of

Fig. 3 Hyperchromicity of poly d(AT):poly d(AT) in presence of DNA-binding (unwinding) protein. Reaction mixture contained alternating copolymer (8.0  $\mu$ g) and binding protein (78  $\mu$ g) in 1.2 ml buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM mercaptoethanol, 20 mM NaCl, 30% glycerol).  $A_{260}$  determined with a recording Gilford spectrophotometer at 24 °C immediately after mixing of copolymer and binding (unwinding) protein at 24 °C. Cuvettes containing copolymer or binding protein alone in buffer were also scanned to calculate increase in absorbance.



RSV RNA in the presence of gene 32 protein<sup>18</sup> Probably due to the collapsed RNA structure, a disturbing feature of reverse transcriptase reaction *in vitro* has been the relatively small size (5–7S) of the product and the faster initial rate of DNA synthesis<sup>20–23</sup>. In the presence of the binding protein, however, the rate of synthesis and the size of the DNA product would be expected to increase. In our preliminary studies using RSV 70S as template, increasing amounts of the binding protein stimulated linearly the rate of DNA synthesis in a homologous reverse transcriptase reaction (Table 2). A detailed study of the influence of the binding protein on the size of DNA products and the kinetics of synthesis is under way.

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## Eukaryotic DNA replication complex

We postulate that eukaryotic DNA replication occurs at discrete intranuclear macrostructures, replication complexes, which might be separable from the bulk of nuclear material. A complex composed of DNA, protein and other nuclear constituents could have a different intrinsic density from nucleic acids or protein alone, and this might be the basis for its isolation. Replication complexes would characteristically contain nascent DNA<sup>1–4</sup> and DNA polymerase. If they are functionally intact, they should also have the intrinsic capacity to synthesise DNA. We have used a previously reported method for the fractionation of sonically disrupted nuclei on gradients of  $\text{Cs}_2\text{SO}_4$  (ref. 5). The results indicate that replication complexes may be partially purified by this method, and that ATP-dependent DNA synthesis is a characteristic of the complexes.

Isolated nuclei from regenerating rat liver were sonicated carefully and the sonicate was banded in equilibrium density gradients of  $\text{Cs}_2\text{SO}_4$  (ref. 5). Three principal bands were observed in the gradients: light band (LB), middle band (MB) and heavy band (HB) (average densities: 1.21, 1.26, and 1.32  $\text{g cm}^{-3}$ , respectively). These bands possess different relative concentrations of DNA, RNA and protein<sup>5</sup>. To demonstrate nascent DNA, rats were labelled with tritiated thymidine for 5, 30 or 120 min *in vivo* 23 h after partial

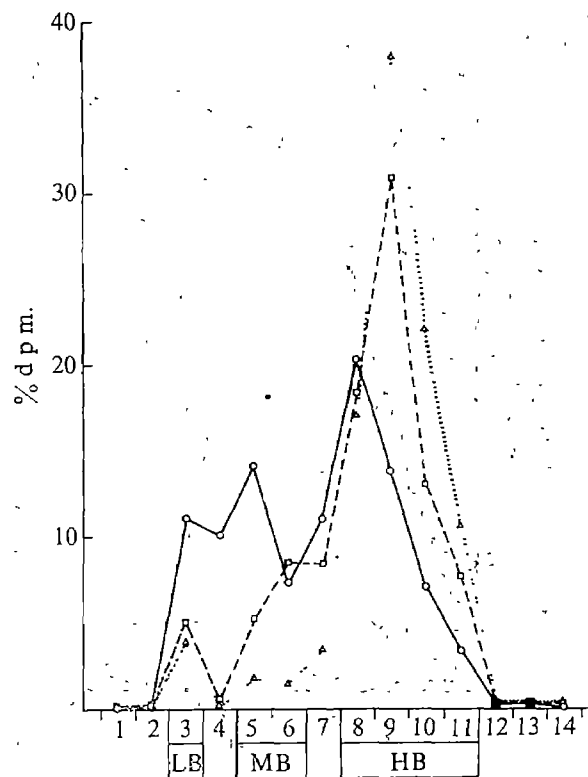


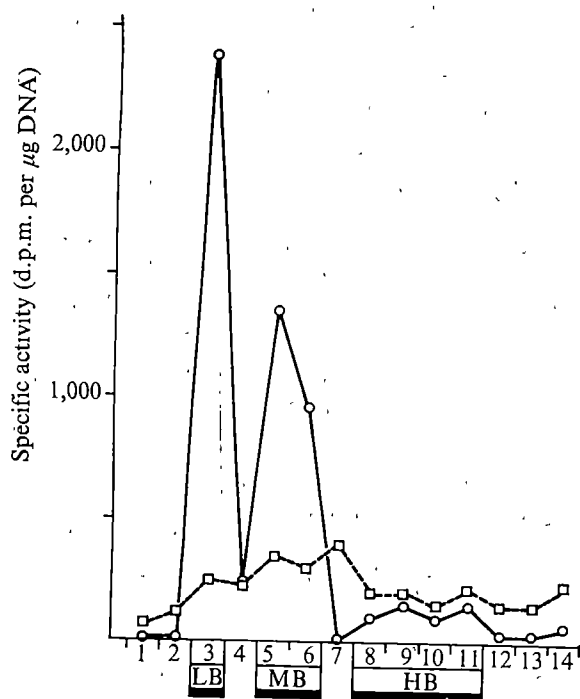
Fig. 1. Distribution of radioactivity in DNA of nuclear sub-fractions after 5, 30 or 120 min of labelling *in vivo*. Female Osborne-Mendel rats weighing 120 g were injected intraperitoneally with tritiated thymidine 23 h after partial hepatectomy. Nuclei were isolated from the livers by the method of Lynch *et al.*<sup>10</sup> Nuclear pellets were suspended in 5 mM Tris-HCl, pH 7.5 containing 5 mM Na<sub>2</sub>EDTA and then sonicated in precisely defined conditions<sup>5</sup>.  $\text{Cs}_2\text{SO}_4$  was added to this suspension to achieve a 1.3  $\text{g cm}^{-3}$  solution, and nuclear constituents were allowed to concentrate at their characteristic intrinsic buoyant densities in the gradients generated during a 66-h centrifugation at 35,000 r.p.m. in an SW40 rotor at 25 °C. The gradients were fractionated into 1-ml portions, and processed as follows: precipitating and washing with cold dilute TCA and PCA to remove  $\text{Cs}_2\text{SO}_4$  and unincorporated radioactivity; treatment with 0.3 M NaOH at 37 °C for 1 h followed by further washing with acid, and hydrolysis of DNA with 1.2 M PCA at 60 °C for 1 h. Radioactivity and DNA content were quantified in this hydrolysate. Nearly all radioactivity recovered after our standard processing was present in DNA and in thymidine residues when this DNA was subjected to enzymatic hydrolysis and chromatography (unpublished observation). The percentage of the total gradient d.p.m. present in each fraction was plotted to demonstrate the distribution of radioactivity after 5 (○), 30 (□), and 120 (△) min of labelling. The total d.p.m. incorporated were 129,191 at 5 min, 357,586 at 30 min and 1,749,712 at 120 min. The portions of the gradients corresponding to the light band (LB), middle band (MB) and heavy band (HB) are marked at the bottom of the figure.

hepatectomy (DNA synthesis peak<sup>6</sup>). The percentage distribution of radioactivity incorporated into DNA of gradient fractions changes with duration of labelling (Fig. 1) and this suggests a precursor-product relationship. LB (which contains 4.9% of the total DNA) has 11.1% of the radioactivity incorporated into DNA after 5 min and this decreases to 3.9% after 120 min; MB (which contains 7.9% of the total DNA) has 21.6% after 5 min and only 3.4% after 120 min; in contrast, HB (which contains 86% of the total DNA) has 44.9% at 5 min and this increases to 88.0% at 120 min. Much of the incorporated radioactivity is found in a small fraction of the total DNA after a short labelling period. With time there is a reduction in the proportion of radioactivity associated with the MB and LB DNA, and an increase in the proportion of radioactivity associated with the bulk DNA.

Previous reports<sup>1–4</sup> indicate that nascent eukaryotic DNA

is partially "destabilised" and seems to have partial single-stranded character because of its ease of denaturation. To evaluate this property, DNA was purified from the  $\text{Cs}_2\text{SO}_4$  gradient bands by rebanding in  $\text{CsCl}$  gradients. The purified DNA was then analysed by hydroxyapatite chromatography<sup>7</sup> and gel electrophoresis<sup>8</sup>. For both LB and MB, a sizeable proportion (15–20%) of the DNA labelled for 5 min *in vivo* demonstrated limited mobility in acrylamide–Agarose gels, consistent with the limited mobility demonstrated for replication forks from *Escherichia coli*<sup>9</sup>. In addition, a larger proportion of LB and MB DNA than HB DNA was more readily eluted from hydroxyapatite than native double-stranded DNA, which suggested a greater partial single-stranded character to LB and MB DNA.

The intranuclear localisation of the labelled product of DNA replication and repair *in vitro* in isolated rat liver nuclei was evaluated in  $\text{Cs}_2\text{SO}_4$  gradients. DNA replication (semi-conservative) in isolated S-phase liver nuclei is ATP dependent<sup>10–11</sup> whereas DNA repair (non-semi-conservative) is ATP independent and best observed in non-S-phase nuclei<sup>11–12</sup>. Analysis of  $\text{Cs}_2\text{SO}_4$  gradients of sonicated nuclei after DNA replication *in vitro* showed the labelled DNA product of the ATP-dependent process to be largely confined



**Fig. 2** Distribution in  $\text{Cs}_2\text{SO}_4$  gradients of DNA product of semi-conservative and non-semi-conservative DNA synthesis *in vitro*. Nuclei were isolated<sup>10</sup> from normal rats and from partially hepatectomised rats 23 h after operation. Nuclei from normal liver were treated with DNase I to stimulate non-semi-conservative, ATP-independent DNA synthesis<sup>11</sup>. Regenerating liver nuclei were incubated with and without ATP in conditions previously used to demonstrate ATP-dependent DNA replication<sup>11</sup>. For non-semi-conservative DNA synthesis DNase-treated normal liver nuclei were assayed in the same conditions, but with ATP omitted. After 5 min incubation at 37 °C, nuclei were cooled in an ice bath, pelleted by centrifugation at 600g and then resuspended in 5 mM Tris-HCl, pH 7.5 containing 5 mM  $\text{Na}_2\text{EDTA}$ . Nuclei were sonicated and the sonicates were banded in  $\text{Cs}_2\text{SO}_4$  equilibrium density gradients<sup>5</sup>. The gradients were then fractionated into 1-ml portions and the fractions were processed as described in the legend to Fig. 1. DNA content and radioactivity were determined for each fraction. ATP-dependent DNA replication in regenerating rat liver nuclei was determined as the activity of nuclei incubated with ATP minus the activity without ATP<sup>11</sup>. The d.p.m. in each fraction were normalised to the DNA content of the fraction and the specific activities were plotted for ATP-dependent DNA replication (○) and DNase I-activated DNA synthesis (□). The total d.p.m. was 76,800 for ATP-dependent DNA replication and 44,800 for ATP-independent DNase-activated DNA synthesis.

to the MB and LB (Fig. 2). In contrast, the labelled DNA product of non-semi-conservative DNA synthesis in isolated nuclei roughly paralleled the total distribution of DNA in the gradient and thus did not show specificity of localisation (Fig. 2). In previous experiments<sup>7</sup> we have shown the presence of DNA polymerase activity (primed with exogenous activated DNA) in gradient fractions of unlabelled liver nuclei and have shown the greatest quantities to be in MB and LB. In other experiments gradient fractions from unlabelled regenerating rat liver nuclei were evaluated for endogenously-primed, ATP-dependent DNA synthesis in assay conditions used for comparable nuclei. In the approximately 50% of these studies in which ATP-dependent DNA synthesis was detected in the gradient fractions, the MB was consistently shown to have the highest levels of this activity (unpublished observation).

The presence of ATP-dependent DNA synthesis and partially single-stranded, rapidly labelled DNA containing replication forks in the same fractions from  $\text{Cs}_2\text{SO}_4$  gradients indicates that this procedure partially purified eukaryotic DNA replication complexes from S-phase rat liver nuclei. This result extends the observation of ATP-dependent DNA replication in isolated mammalian cell nuclei<sup>10,11,13</sup> and complements the observation of ATP-dependent DNA synthesis in MB fractions isolated from sea urchin embryo nuclei<sup>14</sup>. Since nuclear membrane is largely confined to the LB (unpublished observation), the result reported here differs from studies involving the MB technique<sup>14–16</sup> because a sizeable proportion of nascent DNA is in the MB as well as in association with the nuclear membrane (in the LB). The property of ATP-dependent DNA synthesis seems to provide a characteristic *in vitro* assay with which the replication complex can be purified. In future experiments we will attempt to determine whether other enzymes, such as deoxyribonuclease, DNA ligase, DNA-dependent RNA polymerase, RNase H, as well as DNA-unwinding protein are intrinsic constituents of the DNA replication complex. Identification of constituents of the replication complex and preparation of appropriately labelled antibodies to them may allow unambiguous localisation of DNA replication sites in the nucleus by direct visualisation.

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## Evidence for defined lengths of DNA replication units in 'satellite' DNA from *D. ordii*

EUKARYOTIC DNA can be isolated as long fibres on which replication has occurred at several locations<sup>1-9</sup>. These replicating units are arranged in tandem and spaced at intervals which vary in length from 1- to 100  $\mu\text{m}$ <sup>2-5, 7-9</sup>. Although it can be determined that at any time replication has been initiated from a particular position on a fibre, it is not known whether that position remains fixed from one round of replication to the next, nor whether replication terminates at fixed locations on the DNA fibre. Attempts to resolve these questions have used experiments in which replicated units are visualised and their length and spatial distribution reconciled with models of fixed<sup>4,5</sup> or varying<sup>3,8</sup> sites of initiation and termination. Different studies have reached the different conclusions that these sites are variable<sup>3,8</sup> or fixed<sup>4,5</sup> (particularly with respect to termination of replication). All of these studies have been complicated by the observation that the length of replicated regions seems to vary in a manner consistent with a broad but continuous distribution

for the length of the replicating unit. Such a distribution could reflect variation in the position of fixed termination sites or the fact that termination is not at a fixed position on the DNA fibre.

We have isolated and visualised a set of DNA fibres in which the length and spacing of tandem-replicated units are distributed as multiples of a single fundamental unit. These measurements were made on 'satellite' DNA of *D. ordii*<sup>10,11</sup> which had been isolated by CsCl centrifugation. The material to be described was prepared in a series of experiments reported previously<sup>5</sup>.

We have already presented evidence that satellite DNA with a buoyant density heavier than that of main band DNA<sup>10</sup> and characteristically replicated late in S phase<sup>11</sup> (6 to 8 h after release of fluorodeoxyuridine (FUDR) block) can be visualised as multiple short segments of replicated DNA arranged in tandem and interspersed with non-replicated material<sup>5</sup>. In that study we prepared DNA from cells which had been continuously labelled with <sup>3</sup>H thymidine for 8 h after release from FUDR inhibition of DNA synthesis. This DNA was fractionated by CsCl centrifugation and autoradiographs prepared from the different fractions. An outstanding characteristic of this material was the pattern of very long DNA fibres with small replicated and non-replicated regions occurring as tandem arrays on one fibre. We have quantitatively re-examined the material from the densest of these satellite fractions and obtained the distribution of non-radioactive and radioactive regions shown in Fig. 1a and b. A detailed description of the isolation and preparation of this DNA has been published<sup>5</sup> and a summary of this experiment is presented in the legend to Fig. 1.

Both non-radioactive (Fig. 1a) and radioactive (b) regions are grouped into distinct size classes which seem to be the same. That this is the case is apparent when the data for both replicated and non-replicated material are combined (Fig. 2a). In this larger mixed population the discrete length classifications are even more apparent.

It is interesting that the frequency of any particular size class seems to decrease as an exponential function of its size Fig. 2b. This relationship is apparent for all of the more heavily populated classes. The distribution in Fig. 2a was compared with the null hypothesis that pieces are distributed as a continuous function of their length according to the equation: number of any length,  $L = Ae^{-\lambda L}$ . The probability of obtaining the result in Fig. 2a assuming the null hypothesis to be correct is  $P < 0.001$  ( $\chi^2 = 93$ , 27 d. f.). These data are based on a sample of 350 units distributed in tandem arrays on 31 fibres isolated after lysis of a population of  $10^5$  cells and fractionation of the DNA on a CsCl density gradient. During this process, the sample was thoroughly mixed and it is extremely unlikely that any two fibres were isolated from the same cell. A fraction of the same relative density isolated from cells labelled for 10 h instead of 8 h, did not yield tandem arrays, but only long stretches of continuously radioactive material<sup>5,11</sup>. This suggests that the tandem arrays found at 8 h are due to replicated (radioactive) and non-replicated (non-radioactive) regions and that the non-radioactive regions are replicated between 8 and 10 h.

The data in Figs 1a and b and 2a are readily explained by assuming the existence of tandemly arranged replication units of defined length only some of which have been replicated after 8 h incubation in label.

The distribution of sizes is interesting. The size classes approximate one-, two-, four-, six-, eight- and tenfold multiples of a unit the length of which is about 7  $\mu\text{m}$ . Figure 2c compares the distribution of size classes in Fig. 2a with the distribution generated by a computer assuming that size classes are normally distributed ( $\sigma = 3.27 \mu\text{m}$ ) around a mean length which is one-, two-, four-, six-, or eightfold multiple of 6.93  $\mu\text{m}$  assuming a declining amplitude (peak height) equal to  $50e^{-0.037L}$ , where  $L$  is the piece size in  $\mu\text{m}$ . Distributions which fit the data better than that in Fig. 2c can be generated if one assumes that s. d. of each size class is a function of the size, being least for the class of shortest pieces.

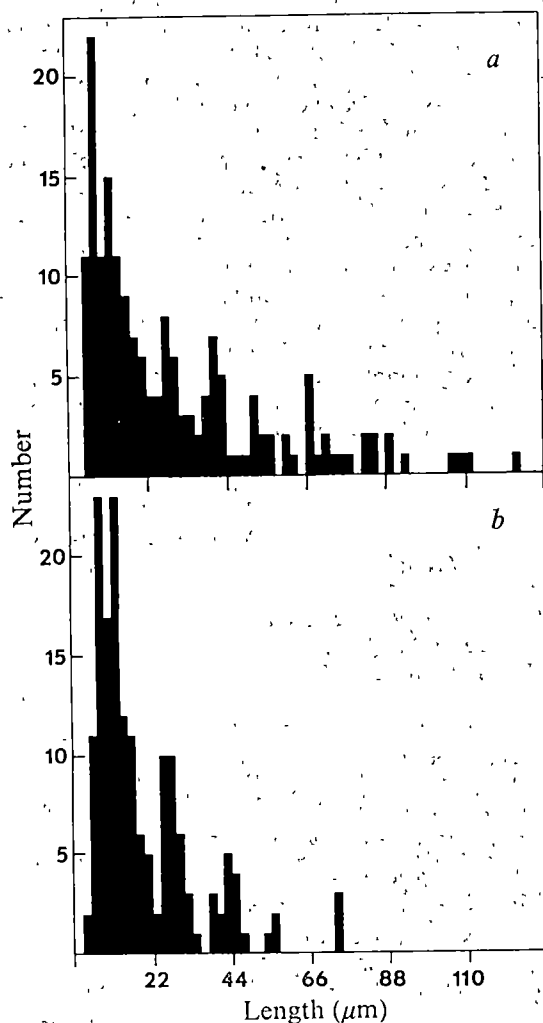
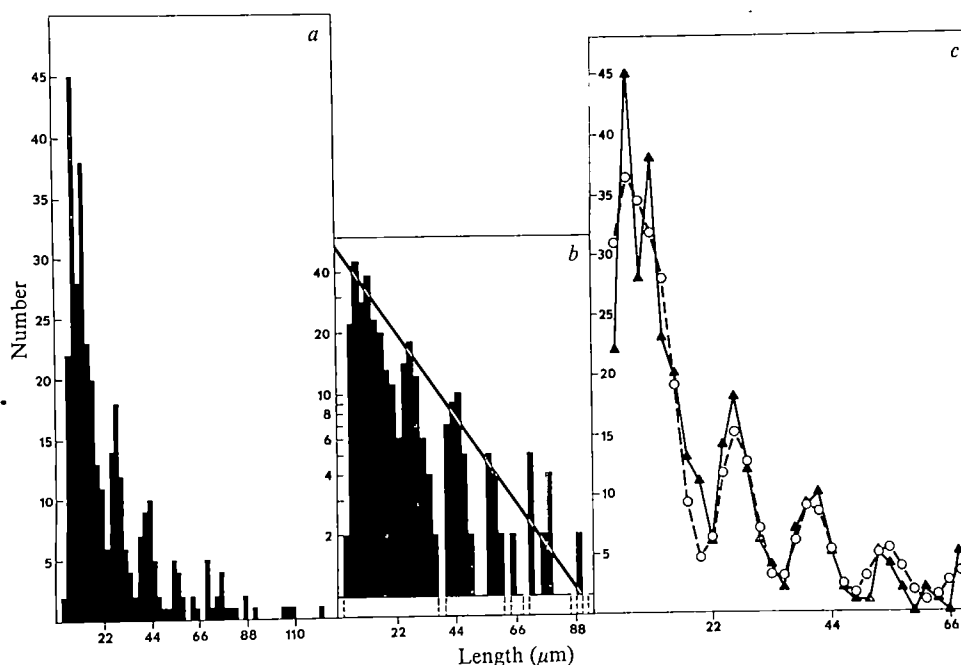


Fig. 1 Frequency of 'a', non-replicated regions (GAPS) and b, replicated (radioactive) regions assorted with respect to length. Cells of *D. ordii* grown as monolayers were inhibited with FUDR ( $1 \mu\text{g ml}^{-1}$ ) for 12 h. Inhibition was reversed by addition of <sup>3</sup>H-thymidine ( $50 \text{ Ci mmol}^{-1}$ ;  $1 \mu\text{g ml}^{-1}$ ) and labelling continued for 8 h. DNA was isolated as described previously and autoradiographs prepared. The density profile of this material has been published (ref 5, Fig. 2). These data were obtained from the fractions designated as S\* in that publication. All of the radioactive units were taken from tandem arrays and only internal units were scored.

**Fig. 2** Combined distributions of replicated and non-replicated regions assorted with respect to length. *a*, linear; *b*, logarithmic frequency scales; *c*, comparison of observed frequency distribution with that generated on assumption that pieces are distributed in classes of one-, two-, four-, six- or eightfold multiples of 6.93  $\mu\text{m}$  (see text for details).  $\blacktriangle$ , Observed frequency;  $\circ$ , best fit distribution generated by computer.



The distribution in Fig. 2*a* and *c* cannot be explained by the simple association of units of the smallest size class because odd multiples are missing. Thus absence of lengths corresponding to three, five or seven times this basic unit requires that the population be composed of at least two categories of units: that corresponding to a single 7- $\mu\text{m}$  unit and those corresponding to groupings of basic units each one of which is approximately twice that size. We have observed that 25% of the smallest units are distributed on two fibres, whereas the rest are distributed on some 17 fibres and are found in every instance as isolated units (that is, 7- $\mu\text{m}$  units, either radioactive or non-radioactive, are separated from each other by larger units). No other clustering of any size class was apparent.

To our knowledge, distributions similar to that in Fig. 2 have not been observed in other material. It seems likely that this distribution reflects the regular spacing of replication units in satellite DNA and that this regularity may be generated by the repetition of particular sequences. On this assumption, it is possible, to construct models which can account for the evolution of the distribution seen in Fig. 2.

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## Low temperature specific heat anomalies in melanins and tumour melanosomes

THE melanins are of great interest because of their widespread presence in biological systems. In humans, melanin is found in nearly all areas where energy transduction occurs, for example, skin, inner ear, retina, as well as in several other areas such as the midbrain. The loss of melanin in the substantia nigra (midbrain) has been correlated with Parkinson's disease, and melanin-drug interactions in the stria vascularis (inner ear) have been implicated in deafness (ototoxicity)<sup>1-3</sup>. Study of the physical properties of the melanins has yielded considerable insight into possible functional roles in biological systems<sup>3-5</sup>, and has led to the design of possible new treatments for the human malignant disease, melanoma, based on interactions between ultrasound and melanin binding drugs<sup>6,7</sup>. These observations have indicated that the melanins play an active, rather than passive, role in biological systems and intensive study of their properties should lead to better understanding of their functions *in vivo*. Such studies are also lending some insight into problems associated with the solid-state physics of amorphous materials<sup>2,5</sup>.

Studies of the physical properties of melanins show them to be highly stable structures, with a large density of free spins ( $10^{18} \text{ g}^{-1}$  (ref. 8)), and also amorphous<sup>9</sup>. These properties seem to be relatively insensitive to the method of preparation<sup>10</sup>. Experiments have shown that melanins exhibit rather exotic electrical characteristics similar to those found in some amorphous structures.

Low temperature specific heat measurements on amorphous solids have led to the observation that in many such materials the temperature-dependent linear term in the specific heat is much larger ( $7-50 \text{ erg g}^{-1} \text{ K}^{-2}$ ) than in the corresponding crystalline state, in which a linear term is negligibly small<sup>11,12</sup>. Data presented here indicate that melanins also exhibit a large linear term ( $50-200 \text{ erg g}^{-1} \text{ K}^{-2}$ ) and that they seem to undergo a phase transition as indicated by the heat capacity near 1.9 K. These effects occur in synthetic as well as natural melanins, but they are most pronounced in the melanosomes isolated from a human melanoma.

Table 1 Low temperature specific heat data for melanin samples

		$\gamma$ (erg g <sup>-1</sup> K <sup>-2</sup> )	$\beta$ (erg g <sup>-1</sup> K <sup>-4</sup> )	$\delta$ (erg g <sup>-1</sup> K <sup>-6</sup> )	$T_0$ (K)	$\Delta C$ at $T_0$ (erg g <sup>-1</sup> K <sup>-1</sup> )
Melanin	1% H <sub>2</sub> O	50 ± 9	120 ± 2	1.64 ± 0.09	1.87	940
Melanin	20% DEA	95 ± 9	99 ± 2	1.84 ± 0.09	1.92	770
Melanosomes	10% H <sub>2</sub> O	220 ± 20	99 ± 4	2.0 ± 0.20	1.95	2,530

All values were determined by data points above approximately 2 K using the normal fitting equation  $C = \gamma T + \beta T^3 + \delta T^5$ .

Melanins can be prepared by the auto-oxidation of L-dopa<sup>5</sup> and also by adding 20% (by weight) of diethylamine (DEA) to the starting mixture of L-dopa and twice-distilled water. Since the melanins exist in the cell as part of a cell organelle, the melanosome, a comparison of the properties of the intact melanosome with that of synthetic melanins was necessary to evaluate the function of the melanins in the intact organelle. The isolation of melanosomes for specific heat measurements was as outlined in Fig. 1. Human malignant melanoma cells obtained at autopsy were used.

Each calorimetric run involved a succession of heat inputs to the sample of a predetermined amount of calories, followed by measurement of temperature increases, using well-calibrated germanium thermometers. The measurements were repeated three times, particularly for the two synthetic melanins, to obtain enough data points for an accurate determination of the electronic specific heat coefficient. The data were fitted to the usual equation  $C = \gamma T + \beta T^3 + \delta T^5$  by the least squares method. The results are shown in Fig. 1 and Table 1, in terms of  $C/T$  as a function of  $T^2$ , where  $C$  is the specific heat in the units of g<sup>-1</sup> K<sup>-1</sup>, and  $T$  is absolute temperature.

The values of the  $\beta$  coefficient in Table 1 indicate that the three materials are quite similar in their lattice contribution to the specific heat. The values of  $\delta$  are also similar but considerably smaller. The linear term  $\gamma$  values, however, are surprisingly large and show a consistent increase as the material

approaches the biologically relevant form. The smallest  $\gamma$  occurs in the highly synthetic melanin, which was polymerised in twice-distilled water. The melanin polymerised with diethylamines shows a higher  $\gamma$  value. (Amines were chosen since they are likely to be present in cellular conditions.) The highest  $\gamma$  value occurs in the biological extract from a human tumour. The increase in  $\gamma$  between the synthetic and the biological material is of clear interest. Although the reason for this is not understood at present, the close agreement in the phonon contributions for the different melanins suggests that the change in the linear term coefficient is unlikely to be due to a change in the phonon spectrum.

An interesting anomalous feature of the data is the observation of a discontinuity at about 1.9 K for all melanins measured in the present experiment. As can be seen in the inset to Fig. 1, this anomaly, though relatively small, is significant beyond the scatter of the data points. The shape of the anomaly seems to be different from that expected from an ordinary Schottky, or  $\lambda$  type, transition. Blois *et al.*<sup>8</sup> measured electron spin resonance for both natural and synthetic melanins and showed the  $g$ -value to be close to 2, and a paramagnetic behaviour following the Curie law over a wide temperature range from 500 K down to 4.2 K. They concluded that the observed paramagnetism was due to unpaired electrons associated with free radicals in the amorphous melanin polymers, rather than an accidental contamination by some ferromagnetic impurities. The presence of

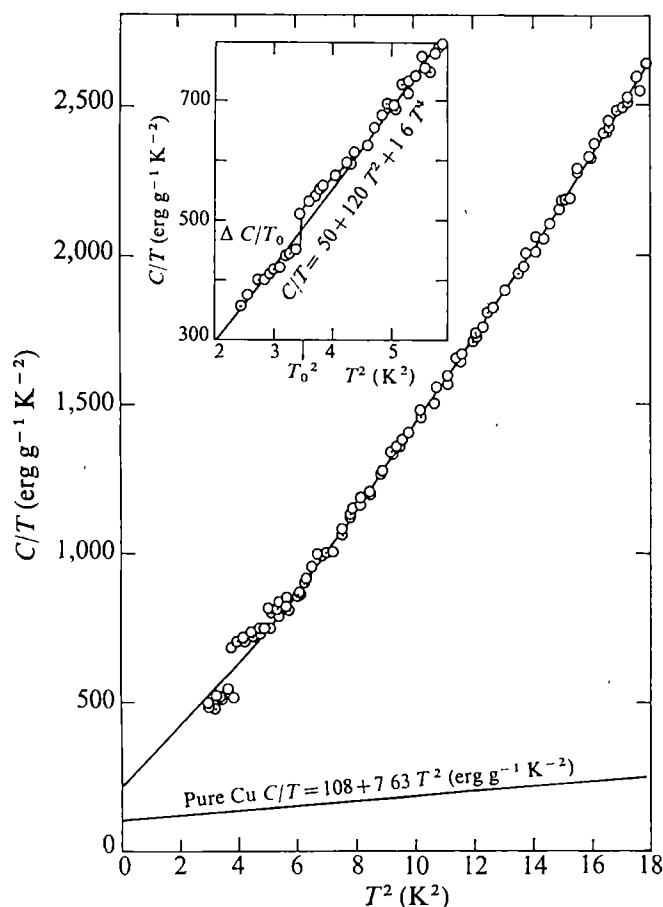


Fig. 1 The usual  $C/T$  against  $T^2$  plot for the melanosome sample. The discontinuous change in the range of 1.9 K is shown for synthetic melanin in the inset. The corresponding plot for copper shows a similar value of the linear coefficient, but substantially different higher term coefficients. The tumour was dissected to remove connective tissue, passed through a fine mesh screen and then vortexed for 15 s in an isotonic salt solution containing 8% sucrose. Phase microscopy examination indicated that most cytoplasmic membranes were ruptured, but most nuclei remained intact. The suspension was centrifuged at 500g for 10 min to remove unlysed cells, cell nuclei and cell debris. The supernatant containing a mixture of cytoplasmic remnants and melanosomes, was spun at 8,000 r.p.m. in a Beckman SW27 Rotor for 20 min. The resultant supernatant was then discarded and the pellet resuspended in 5 ml of an 8% sucrose solution by sonification at about 50 W cm<sup>-1</sup> at 10<sup>4</sup> cycles s<sup>-1</sup>. The material was then layered on an exponential sucrose density gradient (5% sucrose at the top) and spun at 10<sup>4</sup> r.p.m. for 20 min in an SW27 Rotor. The top two-thirds was then fractionated off and the remaining material resuspended and washed twice by centrifugation. The final pellets were found to contain about 3.5 g of material which, in the dehydrated state, was reduced to about 1.6 g, containing approximately 1% water. To prepare specimens suitable for calorimetric work, both synthetic melanins and melanosomes were compressed and cast into cylindrical moulds. After removal from the mould, a thermometer and heater wire were attached directly to each sample. Following the measurement, their contribution to the specific heat was corrected in the usual manner<sup>13</sup>. The DEA melanin sample was also made in the same way as above, but using a small amount of water. After the calorimetric experiment, this water was removed by heating in a vacuum and was found to constitute about 10% of the total weight of the sample. The weights of the synthetic melanins, melanosomes, and the DEA melanin samples were 2.30, 1.68 and 3.20 g respectively. The specific heat was measured in a semi-automatic calorimeter system in the <sup>4</sup>He range in the usual manner<sup>13,14</sup>.

the paramagnetism at 4.2 K strongly suggests that the specific heat data above the anomaly temperature to ( $\sim 1.9$  K) should also correspond to a paramagnetic state, since no specific heat anomaly is observed between 1.9 and 4.2 K. Thus, the anomaly at  $\sim 1.9$  K is probably associated with a magnetic transition, possibly from paramagnetism to antiferromagnetism. The apparently good fit to the equation  $C = \gamma T + \beta T^3$  of the few data points below 1.9 K may indeed be due to the presence of antiferromagnetism in the amorphous melanins. It is also possible that the large linear term is of magnetic origin rather than due solely to the amorphous structure. Antiferromagnetism in amorphous structures has been studied both experimentally and theoretically in amorphous glasses containing some transition elements<sup>15,16</sup> where, however, no sharp magnetic transition was reported.

The melanosome has long been considered a passive cellular organelle. Its considered role as a photoprotective agent in the skin and other illuminated areas, could not explain its presence and function in the non-illuminated areas (for example, the midbrain). A single hypothesis was developed, on the basis of a quantum mechanical model of disordered materials (amorphous semiconductivity) to explain the functional role of the melanosome in both illuminated and non-illuminated areas<sup>2,4,7</sup>. This hypothesis was based on electron-phonon interactions, which seems to be particularly strong in melanins, and on the large density of available energy states.

A particularly useful probe for determining the nature of these states is a measurement of low temperature specific heat. The measurements presented here include two anomalies, a transition and an unusually high linear contribution. The observed anomalies probably arise as a result of the electron-phonon coupling and high density of unpaired spins, which until now were difficult to correlate. Further experimental measurements at near the transition temperature may yield a detailed quantum mechanical description of the states, which will then afford a more precise understanding of the biological functions of melanosomes than has been possible to date.

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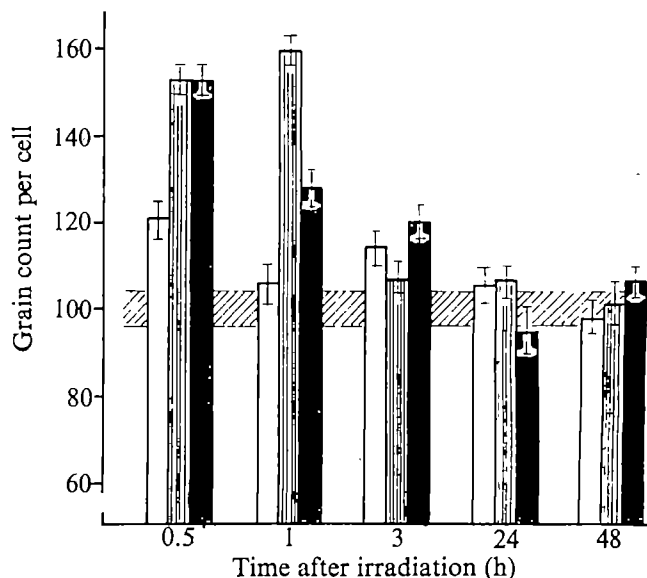
## <sup>3</sup>H-concanavalin A binding of X-irradiated human fibroblasts

STUDIES on the lectin-binding capacity of plasma membranes form a valuable approach for the detection of various alterations in the glycoprotein components of membrane structures. In studying the effect of ionising radiation on the Golgi complex as one of the main structures for glycoprotein biosynthesis or assembly<sup>1</sup>, we wanted to obtain quantitative data for the characterisation of the possible radiation-induced changes of plasma membrane glycoproteins. Using <sup>3</sup>H-concanavalin A (<sup>3</sup>H-con A), specific lectin for glucose- and mannose-containing receptors<sup>2</sup>, we observed a temporary change in the binding capacity of X-irradiated human fibroblasts.

Human WI38 fibroblasts were grown on coverslips in Bellco tubes with 10% heat-inactivated calf serum and 90% Parker solution containing penicillin (100 IU ml<sup>-1</sup>) and streptomycin (100 µg ml<sup>-1</sup>). Cultures were irradiated with a THX250 machine (200 kV, 20 mA, 0.5-mm copper filter, SSD 60 cm). Total absorbed doses were 90, 226 and 905 rad. After irradiation the medium was changed for an unirradiated one collected from cell cultures of the same age. At various times after irradiation the coverslips were washed three times with cold phosphate-buffered saline (PBS) solution and incubated for 5 min at 0 °C (ref. 3) with PBS containing <sup>3</sup>H-con A (1 µCi ml<sup>-1</sup>) (New England Nuclear, 60 Ci mM<sup>-1</sup>). Unbound tritiated lectin was washed out five times from the coverslips with a cold PBS. Coverslips were fixed in Carnoy solution, coated with liquid emulsion by the dipping method (Ilford Nuclear Research Emulsion, Type G5), exposed for 5 d at 4 °C, and fixed and stained with 5% Giemsa solution. Grains were counted in 100 cells for each dose and time point.

In the conditions specified above the number of grains developed in cells were counted. Irradiation resulted in an early (up to 30-60 min) and temporary increase in the amount of bound lectin at the doses applied; the amount of lectin bound to the plasma membrane returned to control level (Fig. 1). Maximum increase was about 60%

Fig. 1 Dose- and time-dependent changes in grain numbers of X-irradiated WI38 human fibroblasts labelled with <sup>3</sup>H-con A. Bars represent s.e.m. Diagonal hatching, control level; open columns, after 90 rad; vertically hatched columns, after 226 rad; solid columns, after 905 rad.





of control. Similar results were obtained when we considered radioactivity per cell, using the liquid scintillation method with labelled cells.

Our result may provide a new way of detecting early structural and functional changes in the plasma membranes because of radiation treatment. The temporary disturbances in membranes which can be detected by this method may lead to elucidation of other phenomena. Further investigations using different labelling conditions for concanavalin and other lectins may also provide promising radiobiological methods or practical tests for the early detection of the extent of radiation injury in animals or humans using their lectin-binding capacities, for example, blood cells. A similar increase in  $^{125}\text{I}$ -con A binding *in vitro* by irradiated (500 rad) porcine lymphocytes was detected after 30 min. Further experiments are in progress to measure the lectin binding capacity *in vivo* and *in vitro* of irradiated blood cells using autoradiographic and liquid scintillation methods.

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## Detection of bound ferulic acid in cell walls of the Gramineae by ultraviolet fluorescence microscopy

THE phenolic acids, ferulic and *p*-coumaric, are bound to cell walls of plants of the Gramineae (which include grasses and cereals) and it was thought that these acids were esterified to lignin and so occurred only in lignified cell walls<sup>1-4</sup>. But it has been shown recently that in cell walls of *Lolium multiflorum* Lam., which contain ferulic acid and a small amount of *p*-coumaric acid mainly as their *trans* isomers, the acids are esterified at their carboxyl groups to polysaccharides<sup>5-7</sup>. The digestibility of grasses by ruminants can be predicted by estimating the amounts of the acids released from the plant cell walls after treatment with a commercial cellulase preparation<sup>8</sup>. The acids could be important in influencing the degradation of plant organic matter in the rumen or in soil and may act as growth inhibitors of plant pathogens<sup>9</sup>. We now report results obtained by ultraviolet fluorescence microscopy, which indicate that ferulic acid is bound to polysaccharides of the walls of a wide variety of cell types of the Gramineae; these walls give a negative phloroglucinol-HCl test for lignin<sup>10</sup>.

Phenolic acids and their ester derivatives often show changes in fluorescence intensity and colour in ultraviolet radiation when treated with ammonia<sup>11</sup>. Ultraviolet fluorescence microscopy of transverse sections (mounted in glass distilled water, pH 5.4) from the middle of an immature internode of *Lolium temulentum* L. showed that all the walls fluoresced blue. This fluorescence was intensified by exposure to ammonia vapour or treatment with 0.1 M ammonium hydroxide solution (pH 10.3), which caused the fluorescence colour of the walls of many cell types to change to green (Fig. 1 and Table 1). This change was dependent on pH and reversed by 0.1 M sodium acetate buffer, pH 4.0. A similar change was shown by carbohydrate esters of ferulic acid obtained from ryegrass cell walls and separated by thin-layer chromatography<sup>5,6</sup>,

suggesting that the walls which fluoresced green contained ferulic acid units esterified to polysaccharides.

Further evidence that the green fluorescence of the cell walls is associated with ferulic acid was obtained by treating sections of immature internode with 1.0 M sodium hydroxide solution at 20 °C for 16 h, which removes esterified phenolic acids from cell walls<sup>1-4</sup>. Cells which previously fluoresced green showed little fluorescence after treatment.

In addition to a negative phloroglucinol-HCl test, the absence of lignin from the walls of most cell types which fluoresced green was indicated by their complete degradation by a crude cellulase (Fig. 2). But the walls of the sub-epidermal sclerenchyma, although they fluoresced green and gave a negative phloroglucinol HCl test, were not degraded by cellulase, apparently because they were partially lignified.

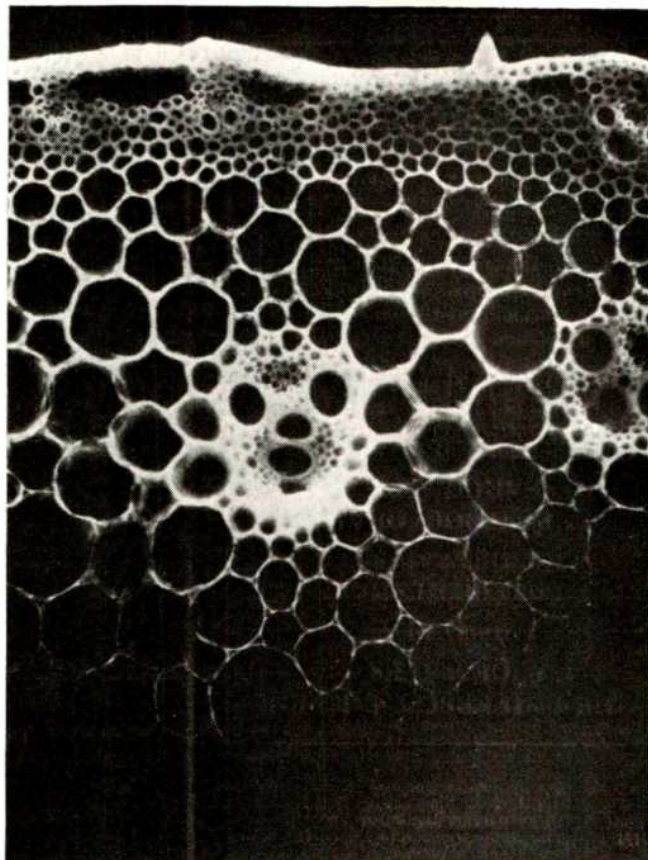


Fig. 1 Transverse section (100  $\mu\text{m}$  thick) from the middle of an immature internode 11.8 cm long (internode below point of insertion of flag leaf) of *Lolium temulentum* L., cut under water using a Vibratome (Oxford Laboratories, San Mateo, California) and mounted in 0.1 M ammonium hydroxide. Photograph taken on a Zeiss Large Universal Fluorescence Microscope (HBO 200 Hg vapour lamp, UGI exciter and No. 41 barrier filters). Plants were grown in controlled environmental conditions (day 23 °C, night 18 °C, light intensity 130  $\text{W m}^{-2}$ , photoperiod 16 h). ( $\times 168$ .)

Cellulase did not alter the fluorescence behaviour of the walls of the cell types which were not degraded. Contrary to earlier suggestions<sup>12</sup>, these observations show that blue fluorescence in ultraviolet radiation does not necessarily indicate the presence of lignin. But those walls which remained blue after treatment with ammonia, and gave a positive phloroglucinol-HCl test, were not degraded by cellulase, which suggested that they were lignified. Some esterified ferulic acid may also have been present in such walls, its green fluorescence being masked by the blue fluorescence of the lignin.

Cell walls of other parts of *L. temulentum* showed fluorescence behaviour similar to that of the immature internode (Table 1). Usually those walls which continued to fluoresce blue after treatment with ammonia gave a positive



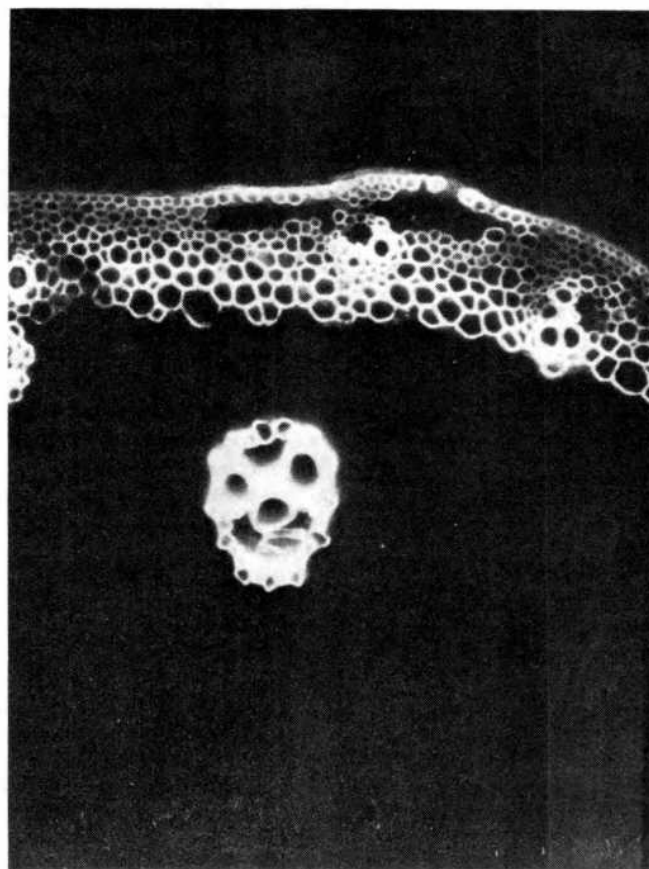
**Table 1** Fluorescence colour of *Lolium temulentum* cell walls of different cell types after treatment with ammonia

Plant part (transverse section)	Blue	Green
Immature stem	Xylem vessels, xylem tracheids and bundle sheath	Xylem parenchyma, phloem, sclerenchyma, epidermis, parenchyma and chlorenchyma
Fully elongated stem	Xylem vessels, xylem tracheids, bundle sheath, sclerenchyma, epidermis and parenchyma (adjacent to sclerenchyma)	Xylem parenchyma, phloem, parenchyma (adjacent to central cavity) and chlorenchyma
Leaf lamina	Xylem vessels, xylem tracheids and inner bundle sheath	Xylem parenchyma, phloem, outer bundle sheath, sclerenchyma (blue middle lamella), epidermis and mesophyll
Leaf sheath	Xylem vessels, xylem tracheids, inner bundle sheath, sclerenchyma and upper epidermis	Xylem parenchyma, phloem, outer bundle sheath, lower epidermis, parenchyma and mesophyll
Glume	Xylem vessels, xylem tracheids, bundle sheath, sclerenchyma, epidermis and parenchyma (adjacent to sclerenchyma)	Phloem, parenchyma (adjacent to chlorenchyma) and chlorenchyma
Lemma	Xylem vessels, bundle sheath, sclerenchyma and outer epidermis	Phloem, inner epidermis and chlorenchyma
Palea	Xylem vessels, inner bundle sheath, sclerenchyma and outer epidermis	Phloem, outer bundle sheath, inner epidermis, parenchyma and chlorenchyma
Root	Xylem vessels, stele fibres, endodermis and pericycle	Phloem, epidermis, cortical parenchyma, root hairs and exodermis
Mature grain	Outer thick walled cells of pericarp	Inner thin walled cells of pericarp, nucellus, aleurone layer and starch cells of endosperm
Coleoptile	Xylem vessels	Phloem, bundle sheath, inner and outer epidermis and parenchyma
Ovary (squash preparation)	No cell types	All cell types
Stamen (squash preparation)	No cell types	All cell types
Pollen (mechanically isolated walls)	Walls of grains	No cell types

test with phloroglucinol-HCl, whereas those that fluoresced green did not. Pollen cell walls, however, although negative to phloroglucinol-HCl, fluoresced blue after treatment with ammonia, probably because of the presence of sporopollenin<sup>13</sup>.

Ferulic acid bound to unligified cell walls seems to be widespread in the Gramineae. This was indicated by a fluorescence behaviour similar to that of *L. temulentum* when transverse sections of the following grasses (leaf laminae) and cereals (immature internodes) were used: *Dactylis glomerata* L. cv. S37, *Festuca arundinacea* Schreb. cv. S170, *Festuca pratensis* Huds. cv. Comtesa, *Lolium multiflorum* Lam. cv. S22, *Lolium perenne* L. cv. S24 and *Phleum pratense* L. cv. S48, *Avena sativa* L., *Hordeum vulgare* L., *Triticum aestivum* L. and *Zea mays* L. cv. Caldera 535.

In contrast to the situation with the Gramineae, ferulic and *p*-coumaric acids or their carbohydrate esters were not released in detectable amounts after treatment of cell walls isolated from young petioles of *Brassica oleracea* L. var. acephala DC. cv. Maris Kestrel (Cruciferae) with sodium hydroxide<sup>4</sup> or cellulase<sup>8</sup>. The fluorescence behaviour of the cell walls of a young petiole of this plant was consistent with the absence of ferulic acid. Only the cuticle and cell walls of the xylem fluoresced in ultraviolet radiation and



**Fig. 2** As Fig. 1, but section treated with a solution of crude commercial cellulase (*Oxytropis* sp., Merck, 2.5 mg ml<sup>-1</sup> 0.2 M NaOH-AcOH buffer, pH 4.8 containing 0.02% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) for 16 h at 37 °C and washed with water. ( $\times 168$ .)



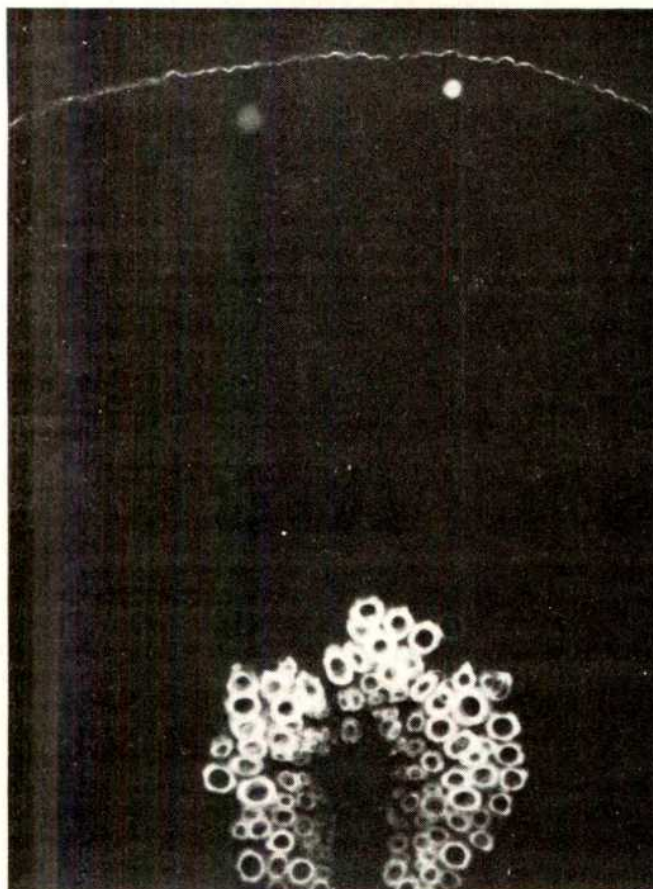


Fig. 3 Transverse section (70  $\mu\text{m}$  thick) of young petiole of field-grown kale. Cut, mounted and photographed as described in Fig. 1 ( $\times 168$ .)

the fluorescence remained blue after treatment with ammonia (Fig. 3). Cell walls of *Trifolium pratense* L. cv. Hungaropoly and *Onobrychis viciifolia* Scop. cv. Cotswold Common (Leguminosae) showed similar fluorescence behaviour, indicating the absence of ferulic acid.

The synthesis and secretion of polysaccharides in the slime of the root cap is analogous to the synthesis and deposition of the matrix polysaccharides in cell walls. The Golgi apparatus and its associated vesicles are considered to be the site of synthesis and transport of the polysaccharides of the root-cap slime, and are believed to have a similar role in the case of the cell-wall matrix polysaccharides<sup>14</sup>. The root-cap slime of *Z. mays* showed blue fluorescence which changed to green after treatment with ammonia, suggesting the presence of ferulic acid esterified to the polysaccharides. It is possible that the site of bonding between ferulic acid and the polysaccharides of slime or of the cell-wall matrix is the Golgi cisternae, the resulting esters being transported within the associated vesicles.

Lignin could be synthesised in the Gramineae by oxidative coupling reactions involving ferulic acid units which are esterified to wall polysaccharides. This is consistent with the finding that the walls of the sub-epidermal sclerenchyma cells and the adjacent parenchyma cells of the fully elongated internode of *L. temulentum* fluoresced blue after treatment with ammonia, in contrast to the green fluorescence of the walls of similar cells of the immature internode (Table 1). Although lignified walls may contain some unpolymerised ferulic acid esterified to polysaccharides, we have shown that as stems of *L. multiflorum* mature, less water-soluble esters are released from their walls by treatment with cellulase.

The work reported here leads us to question conclusions drawn when radioactive lignin precursors were fed to

graminaceous species and the radioactivity detected in the cell walls was attributed to lignin<sup>1</sup>. We suggest that at least some radioactivity is associated with phenolic acids esterified to non-lignified walls. This suggestion is supported by a report that after feeding <sup>14</sup>C-cinnamic acid to wheat coleoptiles, radioactive ferulic acid was released from the walls by sodium hydroxide<sup>15</sup>. The production of vanillin or *p*-hydroxybenzaldehyde by nitrobenzene oxidation of cell walls has been used as a test for lignin<sup>1</sup> but these compounds could be obtained from ferulic acid and *p*-coumaric acids respectively.

We thank Drs L. H. P. Jones and E. L. Leafe for their interest in this work.

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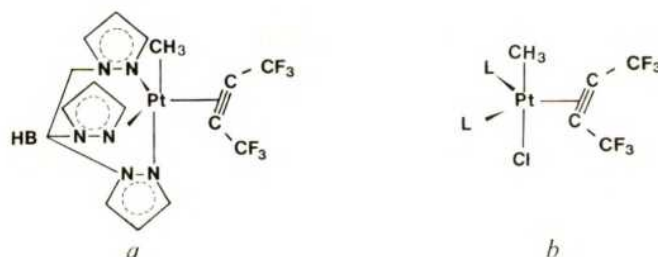
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## Corrigenda

In the article "Mechanism of catalysis of hydrocarbon reactions by platinum surfaces" by G. A. Somorjai and D. W. Blakely (*Nature*, **258**, 580; 1975), the sentence which begins in line 26 of the second column on p. 580 should read . . . The rate of *n*-hexane production per kink site is determined by the slope of the line in Fig. 3b, which gives  $4 \times 10^{-28}$  mol s<sup>-1</sup> per kink atom. This is almost an order of magnitude higher than the slope in Fig. 3a which corresponds to  $5 \times 10^{-29}$  mol s<sup>-1</sup> per step atom.

The sentence which begins in line 10 of the caption to Fig. 3 should read . . . The rates of hydrogenolysis per surface site at the slope of the lines representing hydrogenolysis are  $4 \times 10^{-28}$  mol s<sup>-1</sup> per kink atom and  $5 \times 10^{-29}$  mol s<sup>-1</sup> per step atom.

In the article "Mechanism of reactions at square-planar metal centres" by F. R. Hartley and J. J. Périé (*Nature*, **256**, 636; 1975) Fig. 1a and b are incorrect. The correct forms are reprinted below.



# reviews

## Worthy of sponsored pilgrimage



Ronan Picture Library

Johannes Kepler (1571–1630), an engraving from *Great Astronomers* by Robert Ball (London, 1895).

CENTENARY celebrations of the births and deaths of illustrious men are usually prompted more by an urge to conviviality than by a feeling that history ought to repeat itself at 100-year intervals. Just as it seems to be a law of economics that the rich grow richer as the poor grow poorer, so the great tend to grow greater at the expense of those who are not judged worthy of sponsored pilgrimage and fanfare. The resulting distortion of history will be kept to a minimum only if we stick to a high enough 'fame threshold'—and if Kepler is to fall below our threshold, who will be above it? A volume like that\* now edited by Arthur and Peter Beer, with more than 1,000 pages and encompassing more than 130 contributions, must inevitably include its share of empty eulogy, but the bibliographical advantages of having so much Kepler material in one place are very great—almost as great, in fact, as the difficulty in reviewing it adequately.

The volume takes in the full texts (in English translation where necessary) of the symposia held 400 years after Kepler's birth, in 1971, at Philadelphia, Leningrad, Graz, Linz,

London, Paris, Berlin and Evanston. There are also summaries of other papers, as well as a number of specially written articles published for the first time. The book is richly illustrated—and only Plate 6.4 needs to be read in a mirror. The translations, mostly by Judith Field and Richard Rodman, cast doubt on the editors' plea that translations, like women, are unfaithful when beautiful and not beautiful when faithful. There are 20 sections to the volume, but they fall into four broad groups: Kepler's intellectual influence on contemporaries; studies of his religious, philosophical, alchemical and mystical beliefs; mathematical and physical theories; and other matters which occupied Kepler's thoughts. At the end of the book there are extremely useful bibliographical and iconographic supplements and indices. Altogether, the volume is a monument to the industry and editorial logic of the Beers, father and son.

The short note by D. T. Whiteside, on Kepler's ovals, is an *apéritif* for the invaluable enlarged version of his paper which has since appeared in *Journal for the History of Astronomy*. Another note by Otto Neugebauer, shows that it is hardly surprising to find contemporaries of Kepler failing to understand the import of his work, faced as they were with "rather trivial obstacles . . . on practically every page". The obstacles in question are the trivial computing errors, unexplained changes in parameters, references to (Tycho Brahe's) observations accessible to scarcely anyone else, and so forth. To prove the point in an utterly convincing way, Neugebauer takes the penultimate chapter of *Astronomia Nova*, wherein Kepler shows the need for an elliptical orbit and an equal-area rule.

The *Astronomia Nova* was written in the form of a self-critical intellectual autobiography, and has generally been regarded as an honest one. But as instances of papers showing the circuitous, not to say devious, nature of the path travelled by Kepler's thoughts, one may cite those by Owen Gingerich and E. J. Aiton (three each), as well as that by C. A. Wilson Gingerich, who has written on the same material extensively elsewhere, makes some use of Kepler's unpublished notebooks (now in Leningrad) on Mars. He em-

phasises the importance to Kepler of a three-dimensional approach, and aptly suggests that the idea that the lines of planetary apsides (and hence the planetary planes) should pass through the Sun is important enough to be called Kepler's zeroth law. (The zeroth law is of course logically, if not historically, derivable from the first.) A problem often touched on but rarely tackled with much fervour is that of the interplay of observations (potential and original) and conjecture in Kepler's work. Could the ellipse be observationally distinguished from the alternatives, for example? Curtis Wilson's contribution begins with Newton's assessment: "Kepler knew the orb to be not circular but oval and guessed it to be elliptical". Wilson's all too brief paper explains very clearly in what sense Kepler was indeed guessing. One of the most thorough of recent studies of Kepler's accuracy—in connection with the solar theory—is that reported here in a shortened version of an admirable paper by Y. Maeyama.

Needless to say, Newton was not primarily concerned with Kepler's path to his discoveries. With his "Kepler's Century: Prelude to Newton's", I. B. Cohen starts the volume with, as it were, a Newtonian justification for Kepler's existence, not to mention some entertaining material on later attitudes to the Kepler story.

Outside astronomy proper, the volume includes a number of invaluable sketches which will be of lasting interest to scholars. Jurgen Hübner's assessment of Kepler's theology, J. O. Fleckenstein's of the neoplatonic movement, and Gérard Simon's account of his attempted reform of astrology, for example, help to explain the Kepler who could write (to Mästlin in 1598): "I am a Lutheran astrologer, throwing out the chaff and keeping the grain". Karin Figala considers Kepler's alchemy in the light of a recently discovered funeral oration for the Emperor's personal physician (Martin

\**Kepler: Four Hundred Years*. (Proceedings of conferences held in honour of Johannes Kepler. *Vistas in Astronomy*, Volume 18). Pp. xix + 1,034. (Pergamon: Oxford and New York, 1975). Subscribers' price: \$120.00, £50.00. Non-subscribers: \$150.00, £63.00. Available with Volume 17 on Copernicus. Two-volume subscribers' price: \$140.00, £60.00. Non-subscribers: \$175.00, £75.00.



Ryland II), with a poem by Kepler. This contribution sets higher standards of scholarship than some of the astronomical items. The short papers on Kepler's wanderings from Württemberg to Graz, to Prague, and so on, would make on their own a most enjoyable Baedekker's guide to the manners no less than to the places of Kepler's time.

One of the most surprising things about the 1971 symposia is that so few historians took the opportunity of adding to our understanding of Kepler's optics. A précis of J. A. Lohne's study of Kepler, Harriot, and the sine law is worth noticing, and yet for the full text one must turn to the *Weil der Stadt* proceedings. That a similar remark must be made for many of the articles in this volume is not a weakness but a source of strength. This is

essentially a 'State of the Art' report. In a report within a report, Robert S. Westman considers two early historiographical traditions in Kepler scholarship. In one of these, the history of science was regarded as a useful repository of still valued scientific ideas. The second, hermeneutical, tradition entailed an investigation of the philosophical foundations of Kepler's discoveries. Westman, speaking in Philadelphia towards the end of 1971, placed those papers given to the European conferences against this double historiographical background. This impressive and important volume, in an almost uniformly important series, is therefore complete to a fault, containing as it does its own review. It will be consulted long after its weighty (3 kg) contents have parted company with its covers.

J. D. North

## Applying a technique

*Mössbauer Spectroscopy*. (Topics in Applied Physics, Vol. 5.) Edited by U. Gonser. Pp xviii+241 (Springer: Berlin and New York, 1975.) DM70; \$30.10.

THE increasing use of the Mössbauer technique during the past 15 years in fields as diverse as relativity and archaeology, resulted in the publication of about 1,200 research papers in 1974. Its use in conjunction with more standard techniques in, for instance, chemistry and metallurgy departments means that it is no longer practicable to produce a book giving a critical study of the whole range of applications. Several good introductions to the basic principles exist already but the purpose of the present volume is both to introduce the technique to non-specialists and also to present them with general information on the applications related to their own fields of interest. Thus the introductory chapter is followed by five chapters, written by different authors, concerned with applications in chemistry, magnetism, biology, lunar geology and mineralogy and physical metallurgy. The level seems to be aimed at that of the research worker although the treatment varies from chapter to chapter, with those concerned with chemistry and magnetism requiring familiarity with the methods of quantum mechanics.

In the introduction, the conditions required for the emission of Mössbauer  $\gamma$  rays are described together with the properties of the hyperfine interactions, between the nucleus and its electronic environment, which form the basis of a Mössbauer measurement. A short experimental section is marred by poor

quality spectra and a misleading comparison of  $\gamma$ -ray detectors. Two chapters are devoted to a discussion of the information obtained from the hyperfine interactions, concerning the electronic structure and bonding properties in chemical compounds in general, and in metal proteins. In the latter case the potentially valuable use of the technique to determine the state of the iron in clinical specimens of blood or tissue is mentioned, with the hope that this could lead to advances in medical diagnosis. In the chapter on magnetism there is no mention of work on metals and alloys, and the text is concerned largely with studies of spin orientations in mixed iron oxides. That chapter, particularly, suffers somewhat from being restricted to the isotope iron-57.

The use of the Mössbauer effect as an analytical tool to determine molecular composition is brought out in the chapter on lunar geology and mineralogy, though little discussion is given of the main findings of other measurements. The final chapter on physical metallurgy seems to be at about the right level for an introductory survey. Methods are given for the analysis of the spectra of alloys and intermetallic compounds, with many examples including the study of carbon atoms in steels, the invar problem and the structure of amorphous alloys.

Although the level of presentation varies, in general it is higher than that usually found in an introduction for the non-specialist. As such, the book is more likely to be read by the Mössbauer specialist looking for a presentation of applications outside his own field of study. More comprehensive surveys in each field exist already and the price of \$30.10 is rather high for the purchase of a personal copy.

G. Longworth

## Finite geometries and designs

*Graph Theory, Coding Theory and Block Designs*. (London Mathematical Society Lecture Note Series, No. 19.) By P. J. Cameron and J. H. van Lint. Pp. v+114. (Cambridge University, Cambridge, London and New York, September 1975.) £2.80.

THIS splicing of lecture notes from two courses of seminar lectures given at Westfield College in 1973 is intended for a specialised group of mathematicians, combinatorialists interested in finite geometries and designs, and serves to introduce them to those parts of graph theory and algebraic coding theory that have recently interacted fruitfully with design theory. The authors have, however, provided a brief introduction to design theory proving certain elementary results, stating certain deeper results, and describing many of those designs that arise geometrically. Thus, in principle, the book can be read by any mathematician.

Chapters 2-5 are concerned with the connections between design theory and strongly regular graphs. Chapter 6 is a brief survey of known results concerning so-called "two graphs", a combinatorial structure that arises naturally in the study of doubly-transitive permutation groups. The remaining nine chapters are devoted to those aspects of algebraic coding theory of interest to design theorists. Chapters 9-11 constitute an introduction to coding theory and chapter 15, the last, an introduction to association schemes (a generalisation of strongly regular graphs) and some of the recent work of Delsarte. Sandwiched between are five chapters concerned with those codes of greatest combinatorial interest: Reed-Muller codes, self-orthogonal codes, quadratic-residue codes, symmetry codes, nearly perfect and uniformly packed codes.

The expert will note that proposition 13.4 is incorrect without an added hypothesis and that the remark on page 69 is false since ovals are always seen in the dual. In spite of minor flaws and sometimes sketchy proofs this slender volume should prove useful to its intended audience.

E. F. Assmus, Jr

**Erratum.** The size of the Mayan bas relief shown on p349 of the reviews section (*Nature*, 259, January 29, 1976) was incorrectly stated as  $1.3 \times 1.3$  cm. The correct size is  $1.3 \times 1.3$  m.

## Atmospheres and other fluids

*Atmospheric Thermodynamics.* (Geophysics and Astrophysics Monographs: an International Series of Fundamental Textbooks, Vol. 6.) By J. V. Iribarne and W. L. Godson. Pp. x+222. (Reidel: Dordrecht and Boston, Massachusetts, 1973.) n.p. *Advanced Fluid Mechanics: An Introduction.* By A. J. Raudkivi and R. A. Callander. Pp. xii+325. (Edward Arnold: London, July 1975.) Boards £12.00, paper £5.00.

THE book by Iribarne and Godson, which is based upon undergraduate lecture courses given by the authors, will be welcomed by students of the atmospheric sciences. It is one of the few textbooks in meteorology to deal exclusively with atmospheric thermodynamics and doubtless it will soon become deservedly established as a standard recommended text. The authors assume some knowledge of basic thermodynamics but devote the first four chapters of the book to a review of the fundamental relationships and concepts in the subject. There is some reference to the applicability of these relationships to meteorological problems in the introductory chapters but that aspect is more strongly emphasised in the latter half of the book. Strictly thermodynamic atmospheric processes are discussed at length (fog formation, cloud physics, precipitation, and so on) but sections are also included in which the importance of atmospheric dynamics and radiative processes is emphasised.

Throughout the text one is impressed by the concise and systematic treatment of the subject. Problems are given at the end of each chapter, the diagrams are informative and the book as a whole is well written and well presented. It has a good balance between basic thermodynamics and meteorological applications and will be useful to students and research workers alike.

With students of engineering particularly in mind, Raudkivi and Callander in their book have attempted to produce a work filling a gap which they feel exists between 'elementary' and 'advanced' texts on fluid mechanics. Claiming that the latter have been written almost exclusively by applied mathematicians (Batchelor's "Introduction to Fluid Dynamics" is cited as one example) their primary requirement has been to make the mathematical background simpler and more accessible to students already familiar with elementary fluid mechanics. It will be difficult for the reader to discern any significant improvement upon Batchelor's book in this regard, if indeed such a comparison is appro-

priate. A lengthy section on turbulent flows is included, for example, as is an interesting survey of diffusion and dispersion. The remainder of the text is taken up with chapters on potential flow theory, boundary layers (surprisingly appearing after the section on turbulent flow) and fundamental relationships.

The latter contains much information (rheological models, tides) not usually included in such a section and, as with the rest of the book, it is well written and well presented. Particularly attractive features of the book are the inclusion of experimental data to complement theoretical ideas, and a generous supply of worked examples.

Of necessity, the authors have had to be selective in their choice of topics but they have produced a book which will be of interest to workers in several branches of fluid mechanics.

P. A. DAVIES

## Basic astronomy

*Astronomy Today.* By Fred Hoyle. Pp. 179. (Heinemann Educational: London, October 1975.) £4.50.

*Astronomy Today* is a straightforward basic astronomy text, of the kind which many astronomers could write but which inevitably will reach a larger audience than most through the magic of the author's name. The book is lavishly illustrated and the text is clear, informative and readable. But given its title this is a disappointing book, which describes the Solar System in five chapters, the stars and life in the Universe in one each, and devotes the last chapter to Galaxies and the Universe, presenting a lucid exposition of Hoyle's picture of the redshift as indicating a Universe in which the mass of atoms increases with time.

This presentation of unorthodox ideas in such a way at the end of what is otherwise an orthodox book published in an educational series is curious, to say the least, and it seems that the UK publishers must take the blame for this, since the US edition of the book bears the much more apposite title *Highlights in Astronomy* (Freeman, 1975). The implication that this theory is as well established and broadly accepted as such fundamentals as the causes of seasons and eclipses is most ill-judged in the context of an educational text for young people. And the publishers are further guilty of misjudgement in describing the book on the cover as "ranging from the geological history of the Earth to the latest discoveries about neutron stars and black holes". Black holes are mentioned (once), but only in passing, and there is no mention at all of the state of the art in X-ray astronomy today,

the field in which most of the latest discoveries about black holes and neutron stars have been made.

This is a fine description of "the Solar System today", but in no way is the book representative of the exciting recent advances in high energy astrophysics that the cover and the author's name lead us to expect. It is doubly ironic that not only is Hoyle ideally equipped to write the book we are led to expect, but also that his exposition of novel cosmological ideas would sit far more happily in such a volume.

John Gribbin

## Riboflavin

*Riboflavin.* Edited by Richard S. Rivlin. Pp. xiii+433. (Plenum: New York and London, 1975.) \$46.90.

THIS book comprises a collection of review articles by different workers on the chemistry, physiology and metabolism of riboflavin and the effects of riboflavin deficiency principally in man and rat. Hormonal regulation of riboflavin metabolism, studies on analogues and antagonists and a useful section on methods of assay are well described. Of special interest are the sections on the teratological consequences of riboflavin deficiency and the effect of deficiency on the growth of neoplastic tissue. Editorially the texts have been well cross-referenced and repetition has been minimised without adversely affecting content and readability.

Some abbreviations are used without a preceding definition—M:E ratio and PAH—and inconsistencies occur with others—for example, nicotinamide adenine dinucleotide, NAD (p162), DPN (p308). One section presents arguments for the further enrichment of bread as a means of improving riboflavin status in the US but alternative methods are hardly discussed.

The most serious omission is the lack of adequate detail and discussion on obtaining riboflavin-deficient rats. Chapter 10 mentions briefly (7 lines) the many factors that can influence the deficient state (p305) but without any explanation; and, for the diet used by the author, the reader is referred to a 'recent' reference—1951! This is presumably a mistake, as the diet to which one is referred in no way satisfies the 'severe criteria of deficiency' which the writer considers necessary.

In general, however, the book is well referenced and will provide a valuable and timely addition to the bookshelves of medical schools and institutions of nutrition and pharmacy.

David I. Thurnham

## High fashion in liquid dielectrics

*Simple Dielectric Liquids: Mobility, Conduction and Breakdown.* By T. J. Gallagher. Pp. vi+154. (Clarendon: Oxford; Oxford University: London, October 1975.) £6 00 net.

"BREAKDOWN in liquids could readily become a compendium of unrelated observations", wrote Whitehead in the introduction to his book on breakdown in solids. Although this pronouncement was made from the cosiness of one of those periods when that subject seemed to support an established orthodoxy, it has always held more than a grain of truth. It is the tendency to try to create an orthodoxy which has acted as an impediment to progress in all of liquids research. Arguably, such periods of stasis are little more than a state of mind among referees for scientific journals and other influential workers. The result in a notoriously difficult experimental field is a wild veering of fashion more reminiscent of *haute couture* than scientific progress. Everything in liquid dielectrics has been ascribed successively to ions, electrons, molecular bonds, bubbles or particles, according to vogue.

Technologically this is a very important field. In power distribution alone a small improvement in electric strength would produce enormous financial economies, and in electronics the liquid state has not begun to offer the possibilities provided by the other states of matter. It is an ideal field for PhD training, being generally rather too speculative for industry, yet sufficiently demanding. There has been a large volume of published work which does not seem to be matched by a corresponding degree of solid achievement. Without doubt much of this is due to the nature of the test object itself. The most critical aspect of the dielectric sample, the metal-liquid interface, is almost a completely unknown quantity, yet it determines the transfer of charge in conduction experiments and also the probability of breakdown. It must therefore be disheartening for the new researcher to find a turgid literature rife with ill-controlled experiments and glib theories.

What can one say of an author who has the temerity to attempt to survey such a field? Gallagher has done as good a job as could possibly be expected. This review should rapidly become the *vade-mecum* for all researchers in the field, new or old. It is particularly stimulating to be able to pick out the high points in which one result condemned great chunks of the preceding literature; for example the discovery of the oxygen effect in breakdown, the e.h.d. stability theory, fast carriers in

pure liquids, the statistical nature of breakdown, and so on.

If one has to find the conventional adverse criticism, it is that the author maintains his excellent disinterestedness to a fault. Having made a remark which invalidates a theory (for example, that simple equilibrium thermodynamics cannot be applied to a transient irreversible process such as breakdown) he proceeds to give it a full treatment at the expense of others which might deserve more of his limited space. A similar remark applies to some of the experimental results (for example, the use of strange techniques of statistical selection possibly inspired by vision of the Holy Grail of Intrinsic Strength). These are, however, quibbles in respect of a text which deserves to become mandatory for any serious worker in the field. The diagrams, tables, bibliography, author and subject indexes are all excellent. Readers should note that the treatment does not include the proceedings of the most recent (1975) conference, which is published by the Delft University Press. **J. E. Brignell**

## Coding for isoenzymes and haemoglobins

*Haemoglobin, Isoenzymes and Tissue Differentiation.* (North-Holland Research Monographs: Frontiers of Biology, Volume 42.) By C. J. Masters and R. S. Holmes. Pp. xiii+308. (North-Holland: Amsterdam and Oxford; Elsevier Scientific: New York, 1975) Dfl.79; \$32.95.

THE isozyme (isoenzyme) concept, initiated by Markert and Møller 16 years ago, and the accompanying techniques of zone electrophoresis and specific histochemical staining, have led to new insights in many areas of biology and biochemistry. Essentially all enzymes are capable of existing in multiple molecular forms—isozymes—whether they are encoded in different genetic loci (multiple locus isozymes) or encoded in different alleles at a locus (allelic isozymes=allozymes). This book reviews primarily those isozymes (and proteins with specific function, for example, haemoglobins) which are encoded in different but related genetic loci. Two approaches are successfully used in presenting the concepts. The first is a discussion of the molecular, genetic, and evolutionary bases of the more thoroughly documented isozyme systems. The second is the illustration of some of the ways that isozymes can be used as tools to analyse the mechanisms of genetics, physiology, development and evolution.

This is not an exhaustive com-

pendium of all isozyme systems but selectively focuses on those areas of biology (cellular, developmental, genetic and evolutionary) and those enzymes, which represent the authors' areas of expertise. The results of key papers have been summarised (at times critically evaluated) and integrated into a broad contemporary biological context. The condensations of the material, the selected areas reviewed, and the thorough coverage of the earlier literature make this book a useful adjunct to the recently published four-volume proceedings of the Third International Isozyme Conference (Academic, New York) which is a comprehensive collection of original research reports covering almost all areas of biology.

The biological and experimental utility of isozymes is effectively and persuasively presented. The title is somewhat misleading, however, in that it fails to convey the evolutionary emphasis in the first third of the book. This section deals with the evolution of these multilocus isozymes (and haemoglobins), mechanisms of gene duplication, and the utilisation of these isozyme systems to establish phylogenetic relationships. A physiological rationale is often brought forward to explain the divergence in kinetic, physical and other properties of the isozymes as well as the divergence in the developmental and cellular specificity of their syntheses. There is, throughout the book, an excellent blend of evolutionary and developmental concepts. The middle third of the book analyses the differential expression of related genetic loci during development and the physiological relevance of their temporally and spatially specific syntheses.

The last third of the book is devoted to some current problems in isozymology. An interesting analysis is provided of the mechanisms involved in the regulation of isozyme levels, particularly the balanced contributions of preferential synthesis and preferential catabolism; the latter point has often been overlooked when examining isozyme repertoires. In addition, a thorough analysis is made of the subcellular localisation of isozymes and the genetic and molecular basis for the specificity of the positioning of the isozymes within the cell—a topic of profound importance when considering the mechanisms of enzyme regulation. In this last section the authors indicate those areas of isozymology which will probably be the major research directions of the future. This clearly written and well organised overview of many facets of isozymes and haemoglobins should be of interest to biologists and biochemists representing a wide spectrum of research interests.

**Gregory S. Whitt**

*An Introduction to Medical Physics.* By Edwin G. A. Aird. Pp. viii+293. (Heinemann Medical: London, May 1975.) £4.95.

THIS book deals in a straight-forward way with those applications of physics in medicine that are most used. It is broadly divided into "Ionising Radiations and their Uses" and "Other Applications of Physical", roughly 2:1 giving a rather classical, 'measure the dose', hospital physics flavour. It reads easily, however, and in the radiation sections, the clarity and succinct presentation were appreciated. In the second part some of the information could have been made more up-to-date—although this is where the subject is expanding very rapidly, for example, chapter 8 on the use of ultrasonics.

The discussion of physiological measurement is most welcome for it is here that physics and physiology merge. The emphasis, however, is entirely on the technical—as it is throughout the book—with no attempt to show, even in a small way, the conceptual stimulus of physics.

The number of errors detected were small, an indication of the care in both writing and production, although the confusion between longitudinal and transverse orbits of the medium in the propagation of sound waves is too fundamental to go unremarked (p179) and p184) and should be corrected as soon as possible.

Overall, the book demonstrates clearly one facet of the applications of physics in the community. It can be warmly recommended to its author's intended audience—hospital technicians, radiographers and graduate or specialist nurses. For a graduate scientist entering the field it is well worth reading quickly as the introduction it purports to be.

**R. G. Gosling**

*Comprehensive Chemical Kinetics.* Volume 14: Degradation of Polymers. By C. H. Bamford and C. F. H. Tipper. Pp. xv+562. (Elsevier Scientific Amsterdam, Oxford and New York, 1975.) Dfl 200; \$83.50.

THIS series has been a brave and generally very successful attempt to review the whole field, which is loosely described as chemical kinetics. The present volume describes the very complex reactions which cause degradation in synthetic polymers and it is not surprising that the emphasis is on mechanisms rather than detailed kinetics. Indeed, few polymer degradation reactions are well enough understood to enable precise descrip-

tion of the kinetics of the elementary steps. The book has four chapters, classified according to the mode of initiation of the degradation reaction. The first, dealing with thermal degradations contains an excellent introduction to the kinetic models used in describing these reactions, followed by detailed discussions of the behaviour of a wide range of common polymer types. A similar pattern is followed in chapters two and three, which describe degradations induced by high energy radiation and by photochemical methods. The final chapter is concerned with the complex reactions which can occur when polymer degradation occurs in the presence of oxygen or ozone.

## Books brief

As a whole this book is clearly and concisely written and all the authors have done a good job in reviewing their fields. There is much material of interest both to the expert and the beginner. One serious criticism is that the literature coverage does not extend beyond 1971. A publication delay of four years is excessive by any standards and together with the price makes the value of this book rather doubtful, even for specialist libraries.

**N. C. Billingham**

*Films on Solid Surfaces: The Physics and Chemistry of Physical Adsorption.* By J. G. Dash. Pp. xi+273. (Academic: New York and London, September 1975.) \$26; £13.

THE powerful battery of techniques both experimental and theoretical, which have evolved over the past 20 years has enabled the field of surface studies to progress from a state consisting largely of guesswork to one of increasingly detailed understanding. Attempts on the experimental side in the early 1950s to verify the predictions of 'two-dimensional theory' were generally unsuccessful and the apparently confident prediction—that perfect two-dimensional states, for example, crystals, magnetic arrays, superconductors, could not exist—could hardly be questioned. Thanks to the impressive advances in methods such as electron spectroscopy, field-emission and field-ion microscopy, molecular scattering and to the ease with which ultra-high vacuum conditions can be established,

an increasing amount of reliable information on adsorbed films is accumulating. There is an increasing interest in the study of the statistical thermodynamics of physisorption and on the thermodynamics of non-interacting monolayers, which latter relates to the important question of mobility of adsorbed atoms in the formation of surface films. Much of the progress on the experimental side has come from physicists for whom a volume such as the present one will form an invaluable introduction to some of the more chemical aspects of the subject. The introduction to theoretical studies is clearly written and a useful selection of experimental results—limited presumably to keep the overall length in check—gives an excellent view of the present state of the field.

**O.S. Heavens**

*The Heavy Transition Elements.* (A Macmillan Chemistry Text.) By S. A. Cotton and F. A. Hart. Pp. x+272. (Macmillan: London and Basingstoke, September 1975.) Hard cover £8.95; paper cover £4.95.

THIS book is a logical sequel to a cognate volume from the same publishers, which dealt with the 3d transition elements. A first impression is that of the wealth of descriptive inorganic chemistry contained within its covers. Here is a truly up-to-date account of the significant chemistry of these elements, without the loss of older well-established facts.

The choice of structural figures to illustrate chemical points is excellent; and chapters, sections and subsections of the text are well organised and, helpfully, are consistent from chapter to chapter wherever possible. At the start of each chapter there is a table of coordination number against oxidation state for the corresponding 3d element of the group, which gives a useful summary for comparison purposes.

The book, in addition to covering the 4d and 5d elements from zirconium and hafnium through to silver and gold, has excellent chapters on the lanthanides and actinides, and another chapter devoted to metal complexes containing  $\pi$ -bonding ligands.

Although not intended as a work of reference, the bibliography is adequate, and each chapter is referenced with a range of appropriate review articles.

This book is a must for libraries, and very desirable for the bookshelves of individual inorganic chemists.

**Edward Abel**



# obituary

**Detlev Wulf Bronk** died at New York Hospital on November 17, 1975 from complications following a stroke. He was 78 years old. Bronk was one of the outstanding leaders of science in the United States, and his influence also reached out far into the international scene. In the course of his career, he was active and well-known in scientific organisations in Europe, Asia, and Latin America as well as North America.

His initial scientific interest was in electrical engineering and physics, but he soon became committed to biological problems. As a National Research Council (NRC) fellow in the late 1920s, he worked with A. V. Hill at the University of London on studies of the production of heat in muscles and with Edgar Douglas Adrian, now Baron Adrian of Cambridge, on the development of techniques of recording the electrical activity of single nerve fibres.

Because of these interests, he was appointed director of the newly established **Eldridge Reeves Johnson Foundation for Medical Physics** at the University of Pennsylvania in 1929. This remained his base until he left to become president of The Johns Hopkins University in 1949.

During World War II, he became involved in aviation medicine based on a combination of prior interests, which included a period as a naval air cadet in 1917-18. This activity not only carried him to a number of theatres of combat

but involved him in a variety of applied scientific enterprises which made it natural for him to assume the chairmanship of the National Research Council of the National Academy of Sciences (NAS) at the end of the war. At that time the Council was the major working arm of the Academy.

In 1950, he was elected president of the NAS, where he spent a substantial fraction of his time participating as a leader in the very rapid development of science in the United States that accompanied the great growth of government investment in science following World War II. In this connection he served as a member of the National Science Board of the National Science Foundation for fourteen years and was its chairman between 1956 and 1964.

In 1953, he was asked to become head of the Rockefeller Institute for Medical Research, on whose board he had served since 1946. He accepted this post with the understanding that he would be in a position to develop a graduate program in parallel to the postdoctorate educational program which the Institute had supported since its creation early in the century. The Institute eventually changed its name to The Rockefeller University—the first purely graduate university in the United States. He retired as president of Rockefeller University in 1968, but remained exceedingly active in national and international scientific affairs up to the time of his death. In fact, his illness, which came

upon him quite suddenly, took place just a few weeks after his return from the Soviet Union, where he had participated, as a foreign member, in the 250th anniversary celebration of the Soviet Academy of Sciences.

Detlev Bronk was one of the most honoured scientists of our time, having participated and received almost all of the awards in almost all of the activities that lay within his sphere of professional concern. Those who knew him well and worked with him, however, admired him most for his intense interest in individuals as such. He had an enormous circle of close friends and the gift of bringing them together into effective working partnership on countless occasions. This ability served the interests of the NAS exceedingly well, since he employed it, in a most remarkable way, to make that organisation a highly effective advisor both at home and abroad.

Closely related to his interest in people was a profound conviction that the pursuit of science has its own intrinsic rewards which are reflected in its influence on human enlightenment. He understood quite clearly that, while science could serve as handmaiden to the applied arts, it must not become a slave to them. He believed deeply that the freedom of science is one of the essential freedoms which must be sustained if mankind is to become a more perfect reflection of the Creator.

**Frederick Seitz**

## announcements

### Appointment

**Professor R. N. Hazeldine, F.R.S.**, Head of the department of Chemistry at the University of Manchester Institute of Science and Technology, has been appointed to succeed **Lord Bowden** as Principal of UMIST in September 1976.

### Reports and publications

#### Other countries

United States Department of the Interior. Geological Survey Professional Paper 914. *Geologic Considerations for Redevelopment Planning of Managua, Nicaragua, following the 1972 Earthquake*. By Henry R. Schmoll, Richard D. Krushensky and Ernest Dobrovolny. Pp. iii + 23. Professional Paper 907-A, B. *Grade and Tonnage Relationships Among Copper Deposits*. By D. A. Singer, Dennis P. Cox and Lawrence J. Drew and Geochemical Exploration Techniques Applicable in the Search for Copper Deposits. By Maurice A. Chaffee. (Geology and Resources of Copper Deposits) Pp. vii + A11, iii + B26. (Washington, DC: US Government Printing Office, 1975.) [512]

### Person to Person

Scientist going on sabbatical leave to Stanford would like to exchange houses with someone in the Stanford area. House near St Albans, within easy reach of London, has 3 bedrooms, central heating, garage and schools nearby. Available about mid-August 1976 for 12 months (Dr G. R. Banks, National Institute for Medical Research, Mill Hill, London NW7 1AA).

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

United States Department of the Interior. Geological Survey. Water-Supply Paper 1827-B. *Reactions of Aqueous Aluminum Species at Mineral Surfaces*. By D. W. Brown and J. D. Hem. Pp. iv + 48. Water-Supply Paper 2109. *Surface Water Supply of the United States, 1966-70. Part 3. Ohio River Basin. Vol. 3. Ohio River Basin from Louisville, Kentucky, to Wabash River*. Pp. viii + 633. Water-Supply Paper 2150. *Quality of Surface Waters of the United States, 1969. Parts 12-16. North Pacific Slope Basins, Alaska, Hawaii, and Other Pacific Areas*. Pp. xiii + 480. \$3.50. Professional Paper 712-C. *Hydrogeologic and Hydrochemical Framework, South-Central Great Basin, Nevada-California, with Special Reference to the Nevada Test Site*. By Isaac J. Winograd and William Thordarson. Pp. vii + 126 + 3 plates. Professional Paper 756. *Middle Jurassic (Bajocian) Ammonites from Eastern Oregon*. By Ralph W. Imlay. Pp. iv + 100 + 47 plates. \$2.50. Professional Paper 843-A. *Distribution of Gold and Other Ore-Related Bodies in the Oxidized Zone at Goldfield, Nevada*. By R. P. Ashley and J. P. Albers. Pp. iii + 48 + 4 plates. Professional Paper 878. *Evaluation of Ground-Water Degradation Resulting from Waste Disposal to Alluvium near Barstow, California*. By Jerry L. Hughes. Pp. iv + 33. Professional Paper 888. *Tectonic Studies of the Berkshire Massif, Western Massachusetts, Connecticut, and Vermont*. Pp. iii + 106. (Washington, DC: US Government Printing Office, 1974 and 1975.) [812] Bracteatum: A Potential Domestic Source of Codeine. Pp. 16. (St. Louis, Missouri: Mallinckrodt, Inc., 1975.) [812]

nature

February 19, 1976

## Better treaty is worth waiting for

JULES VERNE in *Sens dessus dessous* (1889) reported that the government in Washington was very interested in the possibility of firing a projectile of 180,000 tons which would displace the North Pole by 23°, thus eliminating the obliquity of the ecliptic and thereby seasonal variations in the Sun's elevation. During 1956, Senator Estes Kefauver, the US Vice-Presidential candidate declared that hydrogen bomb tests could tilt the axis by 10°. Neither Verne's nor Kefauver's calculation took into account the Earth's spheroidal shape. When this is accounted for, the displacements in both cases assume more modest dimensions—being reduced by a factor of about 10<sup>11</sup>. "After seventy years", wrote Walter Munk and Gordon Macdonald, in telling this story in *The Rotation of the Earth* (Cambridge University Press), "the government in Washington still refuses to recognise the existence of the equatorial bulge".

This week the United Nations Conference of the Committee on Disarmament reconvenes in Geneva, and environmental warfare will be high on the list of topics to be studied. If denying Moscow (and London) a decent summer every year is less high on the list these days, there still remain plenty of possibilities, however unfeasible they may be at present. They include

- stimulation or suppression of rain, hail, fog, snow and lightning
- generation and guidance of tornadoes and hurricanes
- modification of climate
- diversion or pollution of rivers and ocean currents
- changes in physical, chemical or electrical properties of the atmosphere or oceans
- stimulation of earthquakes and/or oceanic tidal waves
- disruption of natural vegetative cover

Excellent surveys are given by Jozef Goldblat and Bhupendra Jasani in a recent issue of *Ambio* (Vol. IV, No. 5-6).

In the past two years the United States and Soviet Union have been holding bilateral talks on environmental modification, and will be proposing a draft convention. Its crucial sentence runs: "Each State Party to this Convention undertakes not to engage in military or any other hostile use of environmental modification techniques having widespread, long-lasting or severe effects as the means of destruction, damage or injury to another State Party". Environmental modification techniques are later defined to comprise techniques which change the environment "through the deliberate modification of natural processes".

A veritable industry has grown up around the analysis of what is omitted from arms control and disarmament measures, and this convention offers particularly rich pickings. The exercise is helped by the existence of two earlier drafts—one proposed by liberals in the United States Senate in 1973 and given overwhelming support there, the other submitted to the United Nations General Assembly in 1974 by the Soviet Union. The table shows the most significant variations.

The omissions show rather clearly US military thinking on the subject. Environmental modification techniques that

	Military R & D forbidden?	All states protected?	All operations forbidden?	Review Conference after 5 years?
US Senate Resolution (1973)	Yes	Yes	Yes	Yes
USSR Proposal (1974)	'Prepara- tions for use' for- bidden	Yes	Yes	Yes
US/USSR Proposal (1975/6)	No	No, only 'States Party'	No, only those lead- ing to 'widespread, long-lasting or severe effects'	No

are limited in extent, short in duration or not too severe are permitted. Bickering about where the line is drawn is, by the convention, transferred to the Security Council, where the veto can be used. Research and development are permitted, so techniques can be brought to the point of readiness; if any country decides to turn its hostility on another and has a new and remarkable weapon it is unlikely to be too squeamish about ignoring a treaty. It is difficult to know whether to attach any significance to the limitation of beneficiaries to 'States Party'. China has not been in the habit of signing treaties; the two superpowers seem to be keeping their options open in her direction. Finally, the lack of a review conference seems to be the easiest way to allow the subject to go underground once the ink is dry on the treaty.

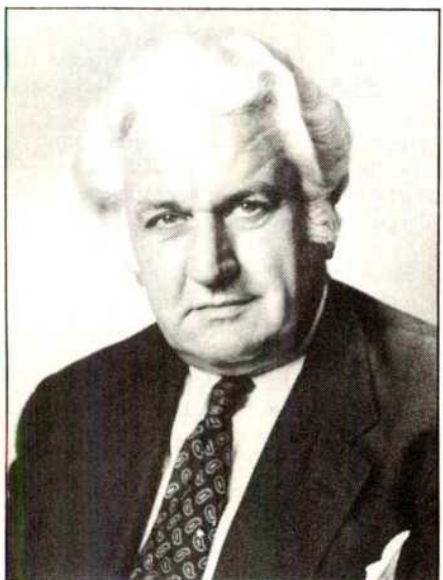
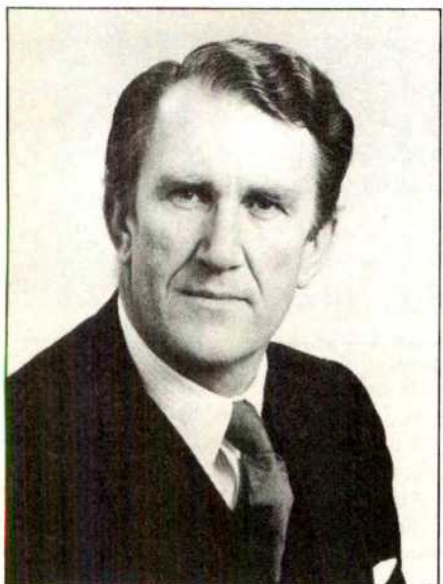
The definition of environmental modification as change "through the deliberate modification of natural processes" was not in the earlier drafts. Was it added simply for neatness or does it exclude anything significant? It does resolve an ambiguity, namely, whether bombing a dam to flood the countryside or illuminating enemy territory at night time is to be regarded as environmental modification. Unfortunately, as with so much else in the convention, the almost feasible is declared beyond its scope.

There are many reasons why the proposed convention is weak, and since widespread, long-lasting and severe modifications are by no means yet upon us (indeed many ideas are by common consent, fairly ridiculous) there is still scope for action. The superpowers should be told to go away and work out something more meaningful in which research and development for peaceful purposes is given a firm international grounding, since the environment does not recognise frontiers. That done, there need be no excuse that military research and development is needed because it might have peaceful applications. And a much more realistic treaty could be written. □



## Australian aftermath

Political changes have affected Australian science. In the first of two articles. **Peter Pockley** paints a picture of the period up to Labour's downfall.



The old, the new and the ever-present: Gough Whitlam (top), Malcolm Fraser (centre) and Sir John Kerr (bottom). (Photos: AIS)

WHILE it may be rash to write historical perspectives close to the events under examination, a claim that Australian science politics have never had a more turbulent twelve months would be hard to beat. The dismissal of Mr Gough Whitlam's Labour government by the Governor General, Sir John Kerr, on 11 November and its replacement, first by vice-regal fiat and ultimately by election of 13 December, by the conservative coalition led by Mr Malcolm Fraser has brought about an atmosphere of tight security and public silence on most areas of government policies and practices. Yet, science politics have boiled merrily along in the public gaze.

All government departments with a substantial science component have suffered change, not because of their scientific responsibilities but largely as a by-product of Mr Fraser's determination to dismantle many of Labour's bureaucratic monuments. However, while the new Prime Minister is working hard to do this, or simply to neglect Labour's social initiatives, like medical and legal aid, he will be hard put to affect one over-riding legacy of the three Whitlam years. This is the evident politicisation of many sectors of Australian society which had previously never noticed, let alone used, their political feet. Under the previous 23 years of Liberal-Country Party rule, the most successful influences on government were the established forces of commerce who operated largely in a private, sometimes covert, fashion. Under Labour, the opportunities for overt political action were expanded, partly by design, partly by default.

The environmentalists and scientists, for example, were encouraged into political action by the deliberate design of direct channels to Cabinet through the establishment of ministers and departments devoted to their interests. It should be noted, though, that these two particular groups and ministries were vastly different. The Environment Department, begun from scratch, had an activist and intellectual in Dr Moss Cass as its first Minister. The staffing of the department had a strong scientific basis from the Secretary, Dr Don McMichael, down. The environmental movement was among the strongest supporters of Labour in their 1972 and 1974 election victories.

In contrast, the Science Department

was formed by splitting the former Department of Education and Science, whose head, Sir Hugh Ennor, went to the Science part. Despite the new organisational design, Australian scientists were slow to forge a stronger platform for their own interests. Some blame was directed at the department which, apart from Sir Hugh Ennor, a former biochemistry professor at the Australian National University, was not strong in scientific experience. The new Minister for Science, Mr Bill Morrison, was less encouraging to the scientists and bureaucrats under his control than he might have been, both through his critical approach to their work and a certain lack of energy in publicly promoting their interests. In an interview for *Nature* in 1973, Mr Morrison remarked that "there are absolutely no votes in science": if true then his time as Science Minister was politically useless to him, but he only needed 30 of the votes which went to his Liberal opponent to have saved his seat at the recent election, and there just might have been 30 disinherited scientists in his electorate.

### Two confrontations

When the government defaulted seriously on science matters, though, the general political climate had prepared scientists for exerting effective public pressure. After a constructive start to 1975 with the announcement in January of the formation of the Australian Science and Technology Council (ASTEC), the government had to ride out two major confrontations with the scientific community in which the scientists displayed considerable nous and had significant effect. The first boilover was the raid on CSIRO's statutory responsibilities by the then Minerals and Energy Minister, Mr Rex Connor (who was returned at the recent poll), following the extraordinary scene of Mr Clyde Cameron's fit of pique in refusing for a time to serve in the Science portfolio. In the face of heavy odds, CSIRO held its ground in the fight.

The second occasion concerned a first-class foul-up of the financing of research grants. There are two principal government schemes for supporting individual and small group research; these are run by the Australian Research Grants Committee (ARGC) and the National Health and Medical



Research Council (NH&MRC). The funds available to these schemes were ruthlessly slashed in the August budget; ARGC's funds for 1976 were cut by 66% to about \$3 million, and NH&MRC's by 43% to about \$4 million. The exact amounts were hard to ascertain from the budget papers which referred to the financial year 1975/76 (July to June) while the research grants schemes, like universities, conformed to the calendar year. The reality of the potential disaster to research in universities and non-government institutions only sank slowly into the collective consciousness of Australian scientists.

The disparate and dispersed nature of Australia's scientific community normally militates against unified and forceful action on a national basis. But, although slow in ignition, the scientists

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**'The disparate and dispersed nature of Australia's scientific community normally militates against unified and forceful action on a national basis. But, although slow in ignition, the scientists were long in burning'**

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were long in burning. In the face of an inflation-conscious government, resolute at last to hold the line on overall expenditure, the scientists mobilised themselves with nationwide protest meetings and lobbying through the press and private channels; one remarkable occasion in Melbourne drew 800 odd researchers—the atmosphere was described as “euphorically united” against the philistine forces of government. By late September, they had forced both Ministers responsible (Science and Consumer Affairs—Mr Cameron — and Health — Dr Doug Everingham, who was later defeated at the poll) to admit to bungling through a failure to appreciate the difference between financial and calendar years during the final budget negotiations. Face-saving formulae were found to restore the 1976 grants to roughly the 1975 levels in paper money terms; for 1976, ARGC finally allocated \$7.2 million and NH&MRC \$7.15 million. Even these levels, though, constituted about 12–15% less in real terms than the previous year due to the effects of inflation.

#### **Prelude to the fall**

By the time the research grants controversy had died down, the Labour Government was beginning to look decidedly shaky. Following the mid-year Cabinet reshuffle, there was firmer and more competent leadership among the senior ministers, but their economic decisions, although basically sound, were biting hard on many important groups. The budget was severe on the

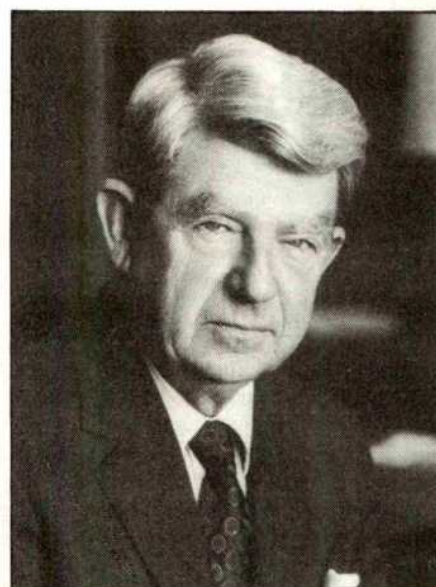
19 universities and 85 advanced colleges by deferring triennial financing for one year. A “pause year” was introduced for 1976, before resumption of the progression of triennia in 1977. The recommendations of the Universities Commission and the Commission on Advanced Education, for expenditure of \$1,780 million and \$1,681 million for the 1976–78 triennium, were set aside, and higher education was thrown into great uncertainty. The effects, though, were mainly felt by the senior administrators of universities and colleges and even their public protests did not cut much ice among their staffs.

The consequences of the economic situation and of Labour's administration of it did not strike home to the great bulk of academics until their own personal interests in research were threatened. In comparison with the cutbacks in growth of expenditure on salaries and capital, the initial research cuts were very small beer (for universities the cutback is of the order of \$200 million for 1976, an exact figure being hard to calculate on a comparative, annual basis because of triennial financing). Yet those initial research cuts, totalling about \$7 million, did more to lose the confidence of the academic community in the government than any other factor, and the government could ill afford to lose the support of any articulate group. Ironically, when the constitutional issue reached crisis point, many of the previously disenchanted academics flocked back to active support of the Labour cause.

It is doubtful if the scientists of Australia ever saw themselves as the precipitants of crisis in government, but their associations with key political events of 1975 were close and, at times, uncomfortably so. By being forced into political defence of their own territory, many scientists appeared to become more informed and articulate on basic political matters than previously.

Mr Fraser chose to use his majority in the Senate to defer Labour's Budget after Mr Whitlam had sacked Mr Connor from the Ministry for allegedly misleading Parliament on an aspect of the long-running “overseas loans affair”. Mr Fraser also took one or two other useful precautions before embarking on this unprecedented course—like squaring off the proprietors of the three national chains of newspapers, whose unquestioning support he enjoyed throughout the campaign.

With the fall of Mr Connor, his adviser Professor Harry Messel disappeared from immediate view in Canberra. The other member of Mr Connor's tightly knit team in the raid on CSIRO, Sir Lennox Hewitt, then Secretary of Mr Connor's Department,



*Top: Sir Hugh Ennor, Secretary, Department of Science. Below: Rex Connor, former Minerals and Energy minister. (Photos: AIS)*

had already departed the Canberra scene, having been strongly encouraged to move sideways into the security of the chairmanship of Qantas, Australia's overseas airline. Nonetheless, the awkward division of responsibility for CSIRO between the Minister for Science and Consumer Affairs and the Minister for Minerals and Energy was translated into a division of CSIRO's funds (\$143 million from all sources for 1975/76) along similar lines in the August budget. Since, however, the budget did not get passed by the Senate until the election had been forced on Labour by the Governor General, the personality of CSIRO was never effectively split by these arrangements.

**(to be concluded)**



## USSR

# Plyushch says protest

*The Soviet Union recently released Leonid Plyushch, the Ukrainian mathematician held for over three years in Dnepropetrovsk penal mental hospital. Vera Rich reports*

"I consider that the scientists of the west should protest in an organised manner and publicly against the campaign in the Soviet Union against dissidents, and against prisons, camps and penal psychiatric institutions". This statement has come from Leonid Plyushch, who warmly acknowledges the help of the western academic community in effecting his release from the hospital following the granting of an exit visa in January. He told *Nature* that he thought pressure from Western scientists was "one of the active factors which assisted my release".

In Plyushch's case, a two-pronged attack on the Soviet authorities was mounted on his behalf—on the one hand by mathematicians and cyberneticists (his professional colleagues), and on the other by psychiatrists, who saw his continued incarceration and the massive doses of drugs to which he was subjected as a blot upon their profession. The mathematicians' campaign followed what has by now become a standard pattern—appeals to the Soviet authorities, proposals to elect Plyushch as a member of eminent mathematical bodies and a concerted effort to keep his case before world opinion. The psychiatrists' campaign was a more complex matter. They were not only concerned with effecting Plyushch's release; they also felt that his committal impugned the honour of their profession. While working to secure his release, they therefore sought a reversal of the diagnosis. This diagnosis stated, apparently on the basis of his criticism of Soviet "state capitalism" (Plyushch's term), his defence of his native Ukrainian culture, and his signature on a petition to the United Nations, that he was suffering from "sluggish schizophrenia" and "reformism"—diseases which, as yet, the psychiatric profession elsewhere does not acknowledge.

Plyushch himself has offered two possible reasons for his committal to the *psikhushka* rather than to an ordinary prison or camp: firstly, he refused to answer the interrogators' questions, "because I considered, and I told them so, that the KGB is an anti-Soviet, anti-Communist, anti-constitutional organisation"; secondly, "because they knew that in court I

would speak from a Communist position and that nothing would happen in court to show that I was an anti-Soviet person". Since it appears to be current Soviet policy to present all separatism in the Union Republics as inspired by fascism and international capitalism, this would have been extremely embarrassing.

Plyushch's ideal for his native Ukraine is an independent socialist state on the Dubcek model—or, at the very least, a reversal of the policy of Russification of higher education and learning, which since the decree on education of November 1958 has gradually introduced Russian as the universal language of the learned professions throughout the Soviet Union. This has in some cases reduced formerly important learned journals into little more than overflow outlets for Moscow publications, and has undermined the very promising lead which, fifteen years ago, the Ukrainian SSR was making in Plyushch's own field of cybernetics.

## Soviet response

In belated response to the campaign by bodies like the Royal College of Psychiatrists, the Soviet authorities, in the person of Dr Snezhnevskii of the Serbskii Institute of Psychiatric Medicine in Moscow, countered finally with accusations of non-professional conduct against the Royal College, which had mentioned "patients" by name. This particular correspondence between the Royal College and Snezhnevskii has not yet been concluded, but it is worth noting that the College only voiced its protests after appeals to world opinion from Plyushch's family and the families of other "psychiatric" prisoners; moreover, an official at the Soviet embassy in Paris had himself divulged names of internees (including that of Plyushch) when he claimed (in a communication to the French mathematician Henri Cartan) that Western psychiatrists who were shown the relevant documents at the Serbskii Institute had all agreed that the persons in question were ill at the time of detention (each of the psychiatrists concerned, who included Dr Denis Leigh, the Secretary General of the World Psychiatric Association, and an ex-President of the American Psychiatric Association, publicly denied this later).

With Plyushch's release, the Soviet Embassy in London has made a determined stand to prove that, even if Plyushch were now sane (as three



Photo: AP

Leonid Plyushch: released

Western psychiatrists—Drs Low-Beer, Sluga and Ferdieri—have declared), he was at the time of his committal mentally ill. *Soviet News*, the official embassy "hand-out", presents his case as a cure; his mental health is said to have "improved lately", "as a result of treatment". His release is thus presented as a triumph for Soviet psychiatry. To reinforce this impression *Soviet News* also carries an interview with Denis Leigh. General in its content, in its present context it could be misconstrued as supporting the Soviet viewpoint on Plyushch.

Although the Plyushch case could now be considered closed, the evidence he has brought with him does reopen certain issues for Western scientists. They have long known of the Soviet use of drugs such as haloperidol, sulfazin and triflazine (stelazin) as a means of repression, and what now emerges from Plyushch's evidence is the number of "patients" involved. Plyushch says that he has "almost no information" about other psychiatric hospitals, and he understands that "things are a bit better" in them. But Dnepropetrovsk, he says, is the worst, and he reports that some 60 dissidents, and not the few originally thought, are at present confined there. He stresses the problem that "in a psychiatric prison, a person degenerates intellectually".

Since it is among the intellectuals that the dissident movement in all its varieties has taken the strongest hold, Plyushch's evidence seems certain to provoke considerable concern and disquiet among the world scientific and academic community as a whole. □

## USA

# The public's case is put

*The tortuous process of setting controls on the use of a revolutionary, but potentially hazardous, technique for manipulating genes from living organisms has entered a new and politically important stage in Washington. Colin Norman reports*

For the first time, the question of controls over genetic manipulation experiments was last week opened up to allow members of various public groups to express their opinions on the risks and benefits of the research, and to influence the development of the proposed controls. The forum was provided by an open meeting of a top-level advisory committee of the National Institutes of Health (NIH), the institution which funds the bulk of biomedical research in the United States. The meeting was called by NIH Director Dr Donald Fredrickson to discuss whether or not the proposed guidelines, drafted by another NIH committee last December after months of argument and confusion, should be adopted. Fredrickson will decide that question before the drafting committee next meets in April. Hanging on his decision are several exciting experiments which are currently under embargo.

The technique involves the use of a newly-discovered class of enzymes to transplant genes from one organism into another. The utility of the technique is that it enables genes from any organism, including man, to be inserted into a virus or bacterium so that they are copied by the reproductive machinery of their new host. That possibility could open up revolutionary advances in understanding how genes work and more distantly, it could lead to such applications as improving yields of crop plants and repairing genetic diseases.

But, in 1973, scientists involved in the research began to express concern about the fact that the technique allows genes to be shuffled between species and joined in combinations which are unlikely to have occurred by evolution. It is conceivable, for example, that a virus or bacterium bearing transplanted genes could be endowed with unpredictable biological properties so that, in the worst imaginable case, a novel epidemic could be let loose if they escape from the laboratory. Such concerns led a committee of the National Academy of Sciences, chaired by Paul Berg of Stanford University, to urge in July 1974 that a

moratorium be placed on some uses of the techniques until the hazards are assessed. That call was followed early last year by an international meeting of geneticists to discuss the hazards; since then, the NIH committee has been attempting to draft regulations to control the technique.

Last week's meeting provided a confused discussion which resulted in conflicting advice being given to Fredrickson about the acceptability of the proposed regulations. The committee, which consisted of scientists, lawyers and lay members, listened to statements from those who drafted the regulations and from a variety of groups and individuals, on the basis of which each member will provide Fredrickson with views on whether or not the proposed controls are acceptable. The fundamental issue is whether, and under what conditions, some of the research which was placed under a voluntary moratorium by the Berg committee's appeal should be allowed to go ahead.

The proposed controls would allow some of the research to be resumed, but under very strict conditions. Essentially, the controls specify that most experiments should be performed in laboratories especially equipped with safety devices—in much the same way that research involving hazardous pathogens is now performed—and, in addition, genes should only be transplanted into organisms which have been crippled in such a way that they would be incapable of surviving outside the laboratory.

Berg himself said last week that he believes that the proposed controls are "stricter than necessary to protect public health", but added that he believes that it is better to err on the side of caution. Similarly, Dr Roy Curtiss, a microbiologist from the University of Alabama, told the committee that he believes the proposed controls are sufficiently strict to guard against the possible hazards. He also announced that he has constructed in his laboratory a strain of the common intestinal bacterium *E. coli*, which appears to have been mutated so as to be incapable of surviving outside an artificial laboratory environment. *E. coli*, the geneticists' workhorse, is the most likely host for transplanted genes.

But a few scientists expressed the opinion that the proposed guidelines are too strict, and will greatly restrict an exciting and potentially beneficial area of research. Dr David Hogness, a member of the committee which drafted the proposed controls, for

example, suggested that there is little evidence that the speculated hazards are real, and argued that "in the present climate of opinion, the benefits of increasing knowledge have been underemphasised". And Dr Donald Brown, a geneticist who has been using the technique to grow and purify frog genes in *E. coli*, suggested that the controls are so strict as to be totally irrational.

On the other hand, several other witnesses, most prominently members of a group of radical scientists called the Boston Area Recombinant DNA group, argued that many planned experiments should be delayed, at least until the hazards have been evaluated. In particular, they urged that *E. coli* should not be used as the host for transplanted genes because that bacterium is a common inhabitant of the human gut.

Dr Allen Silverstone, a spokesman for the Boston group said, for example, that "unless we can be assured that the possibility of danger is reduced to insignificance, we would suggest that the NIH withhold funding such research, until the questions of safety and procedure are settled, especially to the satisfaction of honest critics within the scientific community and the public. We do not propose this lightly. We recognise that many scientists wish to do these experiments, and thus far their restraint has been admirable."

Faced with those conflicting views, Fredrickson must soon decide whether or not to accept the proposed controls or suggest that they should be modified. If he does suggest modifications, it is likely that he will require that the drafting committee should pay more attention to devising ways in which the proposed controls should be implemented.

The proposed guidelines, which would apply only to research funded by NIH, suggest that implementation of the controls should largely be left to institutional committees, which would ensure that the safety precautions at institutions where experiments will be undertaken, meet conditions set out in the regulations.

It should be noted that, by providing public input into his decision, Fredrickson has drawn the sting from a common criticism that the process has lacked participation from people outside the scientific community. Senator Edward Kennedy, for example, has suggested that such matters are too important to be left solely to scientists, and he would presumably be ready to provide such input by legislation if necessary. Last week's public meeting should at least guard against the research being regulated by legislation. □



## SWEDEN

## Probing the ionosphere

*Representatives from six European countries met in Kiruna, North Sweden, last month to inaugurate the EISCAT project for research into the Earth's higher atmosphere. Wendy Barnaby reports*

THE aim of the \$21 million EISCAT (European Incoherent Scatter Facility) project, which scientific organizations in Britain, Finland, France, Norway, Sweden and West Germany are sponsoring, is to learn more about the chemical, physical and dynamic processes which the solar wind induces in the Earth's ionosphere. The dual frequency high power radar system which will be used is expected to be built in three years' time, and the project will run for at least ten years after that Britain, France and West Germany will each contribute about a quarter of the initial financing, and the rest will come from the Scandinavians.

As a method of exploring the ionosphere, incoherent scatter involves beaming radar waves there to cause the free electrons of the ionosphere's plasma to operate as antennae and

'scatter' radio signals, which are then monitored by receivers on earth. These signals are rich in information about the properties of the ionosphere and the processes going on within it. The EISCAT facility will operate from VHF (224 MHz) and UHF (933.5 MHz) transmitters at Tromsø, Norway. The signals will be picked up from electrons at heights between about 80 and 2000 km in the ionosphere by a VHF receiver at Tromsø and UHF receivers at Tromsø, Kiruna (Sweden) and Sodankylä (Finland). They will then be passed to the Geophysical Institute at Kiruna, the project's headquarters, for analysis.

Solar-terrestrial physics will undoubtedly be extended by what the analyses will reveal. One member of the EISCAT Council, Dr F. Horner (UK), has explained that the movement of ionization, the density of electrons, the temperature of ions and electrons, structural changes in the ionosphere and neutral atmosphere, and the ionospheric source of the solar wind will all be further clarified. One of the main topics for investigation will be aurora, and the way in which they

are formed by ionization. The facility is being built in high latitudes specifically to make such studies possible. There will be no immediate practical applications of the research, Dr Horner said, although in the long term it will increase understanding of radio communications. It will also throw light on the ways in which the earth's climate is affected by ionospheric conditions.

Although EISCAT is not the first incoherent scatter project, it is by far the most sophisticated. Teams in Peru and Alaska, for example, have already probed the lower ionosphere with radar waves of 1–10 MHz. The British Science Research Council made the last observations in its project MISCAT (multi-static incoherent scatter) as late as last August; this involved the transmission of waves of 400.5 MHz to obtain electron and ion temperatures in a region 100–400 km within the ionosphere. The data obtained is still being processed at the Appleton Laboratory, Slough, and the University College of Wales, Aberystwyth, and is also expected to yield information about the drift velocities of the ions. For the British scientists, therefore, EISCAT is a logical extension of the SRC's work. □

## BRITAIN

## Engineering a royal society

THE Council of Engineering Institutions (CEI) wheeled on its biggest gun at its tenth anniversary celebration—Prince Philip the retiring president—in the hope of generating badly-needed momentum towards unity in the engineering community.

The Prince, having eloquently exhorted engineers to stick together, launched a new concept—a Fellowship of Engineering, to recognise eminence in the profession; but the idea of a sort of Royal Society for engineers was not without a sizeable band of sceptics.

CEI's problems have multiplied in recent months as engineers, worried about their professional status, their salary, and their public relations, have begun to doubt that CEI was doing enough for them. Meanwhile CEI is suffering from internal strife: its largest members, the Mechanicals, Electricals and Civils, are trying to reform its voting structure, which at present gives equal weight to every one of the fifteen constituent institutions. For lack of progress, the Electricals last December served notice that they would pull out of CEI in a year's time. Unsurprisingly,

the press has now latched on to the woes of CEI in a big way.

Many engineers have looked to CEI to produce some sort of national academy of engineering whose members would be widely recognised as outstanding and whose opinion might be sought by the government. The notion that the government might seek technical advice from professionals not in the Civil Service is a bit outmoded these days, and the need for yet more marks of distinction in engineering doesn't seem entirely obvious when an engineer of distinction can already trail a string of initials like MSc, CEng, FICE, FIMechE, FIGasE, FInstE, MIEE, MICheE after his name. But FEng is now to be the prize to cap all prizes. Excepting FRS, of course. The Royal Society has been a bit of a problem, because it already has seventy or so on its books with at least nominal engineering qualifications. Proponents of the Fellowship of Engineering want very much the FEng to have equal "status" with FRS; and some would even like the Royal Society to stop electing engineering fellows so as to

prevent FEng from being a sort of failed FRS.

There are others, notably the Mechanicals and Electricals, who also worry about the "failed FRS" image through the association of the Fellowship with CEI, "or any body which may replace it". They argue that CEI or its successor will necessarily be of lower technical status, being "mainly concerned with the mundane day-to-day activities of the average member". They would have preferred the Royal Society to have been more extensively involved, preferably as a co-founder and thus as a guarantor of high standards and continuity. This has not happened; invitations have gone out to the 70 FRS's and to 70 other distinguished engineers to become Fellows of the CEI, and the Royal Society simply says that it has learnt of the initiative with satisfaction.

This new mark of distinction thus suffers at the very beginning from dissent about its parent body. This will take a much more serious turn if the Electricals really do pull out of CEI. There will be anxious searching through CEI's mail these next few days in the hopes that a large fraction of the first 140 (the ultimate number is to be 1000) accept their invitations. □

## IN BRIEF

**Reactor action**

Research and technology ministers from France and Germany discussed plans for the joint development of fast breeder and high temperature reactors at last week's intergovernmental meeting in Nice. Both countries are concerned about rising costs, but their research programmes also complement one another well; moreover plans broke down recently for German cooperation with Britain, whose apparent isolation is beginning to show in the face of co-operation on fast breeders between Germany, Belgium, Luxembourg and the Netherlands.

**BNFL setback?**

British Nuclear Fuels Ltd (BNFL), still waiting to close the controversial deal to reprocess spent Japanese nuclear fuel at Windscale, is investigating a potentially embarrassing incident there. Last week, radioactive liquid containing tritium, an element capable of diffusing through apparently impermeable materials, seeped into a stainless steel

pipe carrying cooling water through a storage tank that contains reprocessed material. BNFL claim that no radioactivity escaped from the tank.

**Safeguards draft**

A draft resolution from Britain to fellow members of the International Atomic Energy Agency (IAEA) in Vienna calls for an extension of nuclear safeguards agreements and will, if accepted, come before the IAEA board of governors when it meets next week. Existing safeguards, administered by the IAEA primarily to prevent the clandestine diversion of nuclear materials to military use, apply only to signatories of the Non-Proliferation Treaty; opposition can be expected from non-signatories India and Brazil.

**NASA study**

A major study released by the National Aeronautics and Space Administration (NASA) says that during the next 25 years space research and development

should be directed more toward Earth-oriented programmes, such as environmental monitoring and weather prediction. The study, conducted by a committee of top NASA officials and outside consultants, may form the basis for much of NASA's planning in the next few years, it recommends that space research should focus more on fundamental problems, such as the evolution of the universe and the nature of black holes, that there should be a major increase in emphasis on data management, and that the USA should develop a permanent space station.

**Privacy**

The Data Protection Committee, envisaged in the recent UK White Paper on Computers and Privacy as a first step towards a Data Protection Authority, is to be presided over by Sir Kenneth Younger. It was the Younger Committee's report on privacy in 1972 which first recommended an independent review body to oversee the gathering of personal information.

To obtain support for any piece of research today, it is almost essential to claim that one objective is to produce a model. This use of the word in scientific circles is relatively new. Until recently the Shorter Oxford Dictionary restricted its definition to cover such conceptions as small replicas (like Dinky toys) and to describe those persons who posed for artists and photographers, or who displayed *haute couture* garments to society ladies. The date 1971 is given when it included "a simplified or idealised description of a system, situation or process often in mathematical terms, devised to facilitate calculations and predictions" to the Addenda printed at the end of the second volume.

Provided they are correctly used, some models are clearly useful. One of the earliest was Professor Jay Forrester's world model devised for the long-term study of global problems. Provided it was fed with the correct material, it performed admirably. Unfortunately it could easily be misused, as is often indicated in that egregious publication "Limits to Growth". For instance, it is assumed that there is always an increase in environmental pollution if industrial production increases, and so a growth economy must always lead to intolerable pollution and disaster. There are many arguments against uncontrolled economic growth, but this is probably the least valid. Then it is assumed that DDT levels in fish and fish-eating

birds will rise for many years even after the use of DDT ceases. Fortunately it has been found, in several regions, that almost as soon as the

**Mistaken models****KENNETH MELLANBY**

use of the insecticide is curtailed, levels in fish-eating birds start to fall. These, however, are misuses of what is clearly a true model, and one that can frequently be useful.

My real complaint is that the word model is so often abused. Many so-called models are no more than descriptions—some could best be described as "maps". We even find a written or spoken description called a "verbal model", though not by me,

nor in any publication for which I have any editorial responsibility. Then there is the mathematical model. I read that "in order that it (a hypothesis) can be tested numerically it has to be stated in mathematical terms: this is what is called a 'model' ". Again, not by me. This so-called model is surely nothing but a mathematical description, with all its faults and advantages. I resent the assumption that this is the only way in which to test a hypothesis. Some of us find a mathematical description, no matter how crude (and such a description can only be an approximation to the truth) illuminates the subject, and gives intellectual satisfaction. Others, and I believe they may be just as good scientists, find such treatment merely confusing. The danger today is that the mathematicians, and even the pseudo-mathematicians, may be gaining in influence, and trying to make others adopt methods which may not be the only ones needed to solve all our problems. All descriptions, mathematical, verbal or conceptual, are inaccurate simplifications of the truth, and can never be a substitute for accurate observation.

Recently model has taken on another meaning. Doorbells in the sleazier parts of London are often labelled simply "Model". Some are more descriptive, as "Second Floor, Lulu, Parisian Model, Very Athletic". This does seem a fruitful field for scientific research.



# correspondence

## Egypt's needs

SIR,—Egypt's population, as Salah Galal writes (December 18, page 564), may be expected to reach 220 million by the year 2100, and to satisfy the country's water and power needs in the future would call for two Aswan High Dams a year and the equivalent of two extra Nile Rivers. Sea water desalination is looked to as offering the obvious means of supplying the country's water needs.

This presentation of the situation leads me to ask the following questions:

● Does not the projected vast demand for power and water indicate an unreasonable population growth? Perhaps the problem is not so much how to supply the rapidly increasing population as how to keep the population within suppliable limits.

● The product value of 1 m<sup>3</sup> of irrigation water in Egypt is about 3 cents (US). The cost of producing 1 m<sup>3</sup> of desalinated water by nuclear energy is at least 25 cents. Thus the Egyptian economy will lose 22 cents on every cubic metre of water desalted for use in irrigation, which means a total annual cost of some \$4,000 million for the projected water consumption in the year 2000. Rather than dismaying Egypt's agricultural economists, does this prospect not indicate that a solution to the country's water problem should be sought through other avenues?

As I see it, the most practical answer lies in the more efficient use of the waters of the Nile. It is a fact that a few hundred kilometres east of the Nile Delta, in Israel's northern Negev, the productivity of irrigation water is six times more than in Egypt; the product value per cubic metre of water in that area is 18 cents. Based on this, it may be concluded that the unused potential of the Nile River is itself worth several Niles; and although in order to fully exploit this potential a considerable capital investment might be required, this investment would still be far less than that required for water desalination.

Should it be decided to approach the problem of future resources first and foremost by stepping up the productivity of present resources, I would like to suggest one practical way of contributing towards a solution which would not involve Egypt in inordinate expense: that some of the water draining

from irrigated lands into the sea in the Delta area be sold to Israel.

Every year approximately 10,000 million cubic metres of Egypt's used irrigation water flows through drainage canals out to sea. This water is by Israeli standards still suitable for irrigation. About a tenth of the estimated discharge—equal to the output of dozens of desalting plants—could be absorbed economically in the Israeli Negev and transported there inexpensively by a canal built along the coastal strip of the Sinai Desert. The conveyance of this water to Israel for sale means Egypt's recovery of water that would otherwise be lost.

Egypt's benefits from the transaction would, among other things, be:

● payment for water which at present brings in no economic returns, and  
● a guaranteed amount of food production (in the area in question, with modern irrigation methods, one cubic metre of water produces 10 kilograms of potatoes).

The political climate in the region at present might not appear conducive to the implementation of a project of this nature and, for the time being, the idea put forward here may seem fanciful (although perhaps less fanciful than the projected annual 18,000 million cubic metres of desalinated water by the end of the century). However, even at this stage it might be worth examining the economic advantages of such an undertaking for both participants, since perhaps the very consideration of such a scheme could contribute towards the improvement of the political conditions and help to bring closer a time when such a project might become practicable.

Yours faithfully,

B. KALLY

## Photochemical smog

SIR,—I wish to call attention to a potential hazard in controlling photochemical smog by reducing hydrocarbon (HC) emissions without a corresponding reduction in the emissions of the oxides of nitrogen (NO<sub>x</sub>). If HC concentrations are reduced to keep hourly oxidant levels below the EPA ambient air quality standard of 80 p.p.b., and if NO<sub>x</sub> concentrations are not correspondingly reduced, then on many days all the NO will not be oxidized. In the presence of NO, ozone levels are

always considerably below the normal background level of 25 p.p.b., because of the rapid reaction between NO and O<sub>3</sub>. Thus on the days that NO is not completely oxidised, ozone levels will be considerably below background levels. If many such days should occur in succession, the bacteria count might increase, and this might enhance the incidence of disease (for example, streptococcus salivarius shows 90% mortality when exposed to 0.025 p.p.m. O<sub>3</sub> at 60–80% relative humidity for 30 minutes).

In many communities automobiles account for a greater percentage of the photochemically active HC than of the NO<sub>x</sub>. (In Los Angeles it is about 90% for photochemically active HC, as against 70% for NO<sub>x</sub>.) However the control devices presently being installed on cars are designed to control 80% of the HC and 40% of the NO<sub>x</sub>. As a result the percentage reduction in photochemically active HC should be about 2–3 times as great as the percentage reduction in NO<sub>x</sub>. This may not be a policy of wisdom.

Yours faithfully,

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## Turkish poppy industry

SIR,—A footnote to Peter Collin's article (January 15, page 73): when the Government of Turkey forbade farmers to grow poppies, it became essential to collect substantial seed samples of populations of the many types of poppies grown in the country, both to conserve genetic diversity for future use and to ensure that sufficient seed would be available if it were once again to be required. This was successfully organised by the Crop Research and Introduction Centre at Menemen, Izmir, which was established in 1964 by the Government of Turkey with the cooperation of UNDP and FAO, and whose purposes have always included the conservation and study of the vast resources of genetic diversity in the economic plants of Turkey and their wild relatives (see *Nature*, 258, 278–279, 1975).

Yours faithfully,

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# news and views

## Immunity to parasites

from K. N. Brown

IN a world where a majority of the human population suffer serious morbidity and mortality from eukaryotic parasites, control of these infections must be one of the most pressing of current biomedical problems. Fortunately, there are few more intriguing biological challenges than the capacity of protozoan and metazoan parasites to maintain an integrated existence with their hosts long after being recognised and responded to by the host's immune system. Since they combine this characteristic with an ability to move, as a normal part of their life cycle, between a number of quite different environments, for example, from human blood to mosquito gut, and from fresh water to vertebrate tissue, eukaryotic parasites display an unusual level of phenotypic adaptability.

In the relationship with their vertebrate hosts, parasitic protozoa have the important advantage that every cell (except individual gametes) has the potential to carry the life cycle through to completion. Thus a population can sustain considerable cell death and yet survive as a 'strain'. Because transmission is often intermittent and seasonal however, it is simply not enough for a few individuals to survive host immune reactions, the survivors must themselves multiply in that same immunocompetent environment. In at least three genera, trypanosomes, plasmodia and babesia, this feat is achieved by repeated changes of exposed antigens.

Cross (Parasitology, **71**, 393; 1975) in a careful study of trypanosome variable glycoprotein antigens, has shown very extensive differences in amino acid composition from one variant to another, indicating that, as has long been suspected, variation is a phenotypic event, probably antibody-induced. That differences in the exposed part of the molecule would need to be sufficiently profound to exclude antibody cross-reactivity, could be surmised from the nature of the infection and earlier serological studies, but that they should involve a major part of the molecule came as a surprise.

Whether or not antigenic variation in *Plasmodia* and *Babesia* occurs by changes in analogous surface glyco-

proteins important for extracellular survival, is conjectural, but seems very likely from recent observations by Miller *et al.* (*J. Immun.*, **114**, 1237; 1975).

In any event it is by antigenic variation that these parasites evade some of the worst consequences of the host immune response, and render artificial immunisation extremely difficult. Immunisation by controlled infections still remains the only realistic method of inducing protection, although frequently unsatisfactory and impractical. Thus the recent report of the protective effect of intravenous BCG against murine *Babesia* and *Plasmodia* by Clark *et al.* (*Nature*, **259**, 309; 1976) is important, although the authors' suggestions concerning the role of non-antibody T cell products in destroying intra-erythrocytic parasites, will require much more critical examination before it is generally accepted. The effects, immunological and otherwise, of BCG on host responses to these infections, need investigation.

Many observations have shown that T cells are crucial for protective immunity to malaria. Brown (*Nature*, **230**, 163; 1971) has suggested one interpretation of their role in relation to clinical immunity and antibody responses to successive antigenic variants, and protection has been transferred to non-immunes by T cells from immune animals (Brown, *Ciba Symp.*, **25**, 36; 1974). Elegant experiments by Jayawardena *et al.* (*Nature*, **258**, 149; 1975) using the T<sub>H</sub>T<sub>8</sub> chromosome marker have correlated capacity to produce a protective response to malaria with T cell reactivity.

Parasite survival may be helped by their ability to induce immunodepression, and immune responses to other pathogens may be inhibited (for example see Cox, *Nature* **258**, 149; 1975). This effect seems to be localised to the lymphoid tissue directly exposed to the parasite (Golenser *et al.*, *Protozoology*, **22**, 71A, 1975), and is most readily detected during acute high parasitaemias, although in some particularly virulent trypanosome infections immunodepression may be more generalised. Parasites may produce

nonspecific lymphocyte mitogens (Greenwood and Vick, *Nature*, **257**, 592; 1975). Although responses to unrelated antigens may be depressed, however, specific responses to the primary parasitic infection are extremely active, and the antigenic lability of parasites, rather than shortcomings in host immune responses, seem to account for their survival.

Unlike protozoan populations, individual metazoan parasites cannot sustain any great degree of cell death, but they can maintain intimate contact with host tissues without producing any of the localised cellular reactions which normally characterise the presence of foreign tissues. In schistosomes, profound changes occur in the structure of the parasite surface in the first few hours after skin penetration by the aquatic infective larva. Electron microscopy (Hockley and McLaren, *Int. J. Parasit.*, **3**, 13; 1972) suggests that the absence of host cell reaction depends on continuous membrane activity at the schistosome tegument, especially evident in the first few hours after penetration. Other experiments have shown that antibodies which will kill schistosomules immediately on penetration will not destroy older worms (Clegg and Smithers, *Int. J. Parasit.*, **2**, 79; 1972). The parasite surface is immunogenic, since by injecting TNP-treated schistosomules into infected mice, Ramalho-Pinto *et al.* (*Nature*, **259**, 603; 1976) have shown T cell recognition of surface antigens. Since this response falls later in the infection and cannot be recalled readily by cercarial challenge, it may be significant for survival of adult worms. Immunity to reinfection develops normally in these animals, which is rather puzzling.

Curiously, although adult worms usually avoid immune destruction, many are killed in heavy infections. Schistosomes have host-like antigens, probably glycolipids, associated with their surface, which may mask antigenicity (Clegg, *Ciba Symp.*, **25**, 162; 1974; Smithers and Terry, *Adv. Parasit.*, **14**, 399, 1976). Can the specific T cells demonstrated by Ramalho-Pinto *et al.*, under strong antigenic

stimulus, help B cell responses to these host-antigen-worm membrane associations, to produce antibody-mediated death of adult worms? The semi-auto-immune nature of such a response could require for its initiation a heavy infection with its massive antigenic stimulus.

Clearly in infections with protozoan

and metazoan parasites, the problems of parasite-host integration and host immune responses are complex. A full understanding of interactions and reactions at the interface between parasite and host is essential if we are to be able to interpret and modify host-parasite relationships to man's advantage. □

## Colliding beam fusion

from John G. Cordey

THE controlled fusion of the light elements deuterium and tritium has been the main goal of plasma physicists for the past 20 years. The basic problem is one of containing a mixture of deuterium and tritium at a temperature of  $10^8$  °C for a sufficient length of time so that a significant number of fusion reactions take place. At these temperatures the gaseous mixture is a fully ionised plasma which has to be isolated from metallic walls. The main approach to the containment problem has been to use a strong magnetic field to separate the plasma from the walls. Considerable progress has been made using this technique, and toroidal containment devices of the tokamak type are now being designed, which will have parameters similar to those of future fusion reactors (Rebut, *Phys. Bull.*, **26**, 264; 1975).

The precise conditions for a reactor to break-even on energetic grounds were first given by Lawson (*Proc. Phys. Soc. B70*, 6; 1967). For a plasma in thermal equilibrium (that is with Maxwellian distributions of deuterons and tritons) the Lawson criterion requires that the temperature be greater than 5 keV, and the product of density and the containment time (the ' $n\tau$ ' product) be greater than  $10^{14}$  s cm<sup>-3</sup> for break-even.

The colliding beam torus, discussed by Kulsrud and Jassby on page 541 of this issue of *Nature*, and also by Cordey *et al.* (*Nucl. Fusion*, **15**, 710; 1975) is a toroidal containment device in which the deuterons and tritons, instead of being Maxwellian, are oppositely directed beam-like distributions. These counterstreaming distributions are built up by injecting 60 keV beams of deuterons and tritons into, say, a tokamak device. (This technique of beam stacking has been used for many years in accelerators, at a much lower density of course.)

The mean relative velocity of the two counterstreaming distributions is chosen to be close to the peak of the D.T. fusion cross section at around 120 keV. The resulting fusion power output per

unit volume of the CBT (colliding beam torus), is thereby much larger than that of a conventional fusion reactor with Maxwellian distributions at say a temperature of 10 keV. The conditions for a break-even reactor using the CBT approach are as a consequence far less restrictive. In particular, the ' $n\tau$ ' product has only to be greater than  $3 \times 10^{12}$  s cm<sup>-3</sup> for break-even, rather than the  $10^{14}$  s cm<sup>-3</sup> demanded by the Lawson criteria for the conventional reactor. Since the energy containment time is thought to scale with the radial dimensions of the device, a small scale experiment demonstrating the efficacy of the CBT approach could be built, whereas a demonstration experiment of the conventional reactor will have to be very large indeed. In fact, the next generation of conventional fusion experiments (such as JET, TFTR) are becoming so large and expensive that in Europe they are having to be built collaboratively.

There are however, it seems to me, several possible difficulties associated with this alternative CBT approach. First, the setting up of the colliding beam configuration requires that the containment time of the thermal plasma is less than the fast ion slowing down time, and that there is no recirculation of the thermal plasma. Although as the authors point out, this first condition is satisfied in present-day tokamaks, the parameters in these machines are very different from those of the CBT. In particular, the mean ion energy is much greater than the electron energy whereas the reverse is the case in tokamaks. In view of the fact that the ion distribution in the CBT is anisotropic, there is a strong possibility that the plasma may suffer from microinstabilities which would reduce the slowing down time of the fast ions. The prevention of recirculation of thermal particles relies on the design of an effective diverter; so far there has been little experimental work on diverters and so this part of the proposal remains somewhat speculative.

Also I have some doubts as to

whether the CBT can be developed into an economic reactor in its present form. The quoted values of  $Q$ , the energy amplification factor, of 4-8 are somewhat smaller than the value of 15 which is said to be necessary for an economic fusion reactor system (Conn *et al.*, *Nucl. Fusion*, **15**, 775; 1975). Even if a pure fusion reactor is not possible however, the high power output per unit volume of the CBT means that it would be eminently suitable as a neutron test facility or with a fissionable blanket as a fission-fusion hybrid reactor.

One further problem that is not mentioned in this particular article, but discussed elsewhere by Jassby (*MATT*-1145; 1975), is concerned with the penetration of the injected fast neutrals into the plasma. So that the ions leaving the ion sources can move freely across the containing magnetic field, they are neutralised by a gas cell; on entering the plasma they are then re-ionised by the trapped ions. The mean free path of 60 keV deuterium atoms in a plasma of density  $n$  is  $(2 \times 10^{15})/n$  cm, this places a restriction upon the density or the radius of the device, either of which may affect its reactor potential.

I think to assess whether this novel idea can be developed into a reactor first requires an experimental demonstration of the setting up of the colliding beam configuration. This could probably be done on an existing tokamak with the injection of a few megawatts of, say, 40 keV ions, which is well within the reach of existing neutral beam technology. The scaling of these experiments would then give a clearer indication of the future potential of the CBT as a reactor. □

## Biological safeguards in genetic engineering

from David Sherratt

As well as discussing the types of safeguard needed to eliminate any hazards resulting from genetic manipulation experiments in which genes from potentially any organism can be introduced into and replicated in bacteria, a number of biologists are now attempting to assess experimentally the possible hazards and to develop ways in which they can be minimised (see *Nature*, **258**, 861; 1975). Safeguards fall into two categories—physical containment, which prevents the escape of any possibly hazardous material, and biological containment, which ensures

that any possibly hazardous material can only function in precisely defined conditions unlikely to be found outside a designated laboratory. Suitable physical containment facilities are already available in a few laboratories that handle dangerous pathogens.

Since genetic engineering experiments involve the insertion of the DNA to be cloned into a 'vector' which can then be introduced into a bacterium where it is capable of replication and expression, biological containment can be aimed at both the vector and bacterium. At present the favoured vectors are either derivatives of bacteriophage lambda or one of a number of small plasmids, which consist of duplex circles of DNA able to replicate autonomously within bacteria. The favoured host bacterium is *Escherichia coli*, largely because it has been extensively studied by molecular biologists over the past 20 years.

Unfortunately *E. coli* is a normal inhabitant of the gut, and even though most laboratory strains seem to fare badly when competing with the normal intestinal flora, it has been felt advisable to construct even more 'crippled' strains which not only are unable to grow, or transfer any of their genes in the gut, but are killed by the gut environment. To this end, strains are being constructed that have some or all of the following features:

(1) Nutritional requirements which cannot be met in the intestine, such as requirements for the cell wall constituent diaminopimelic acid and nucleic acid precursors; (2) inability to survive at gut temperature and possession of DNA which is degraded at this temperature; (3) sensitivity to certain gut contents such as bile salts

The introduction of other 'weakening' genetic mutations to the host bacteria (such as nonsense suppressor mutations which upset normal translation and can markedly inhibit cell growth, and temperature-sensitive mutations) may depend on the particular vector used. Since plasmid vectors and their inserted DNA need to be maintained within the host bacterium (naked DNA soon loses its biological activity outside the cell), it is particularly important that the host/plasmid combination is effectively 'crippled' and the presence on the bacterial chromosome or on the plasmid of additional mutations that render the vector unable to replicate or transfer at 37 °C is desirable.

On the other hand, with lambda as a vector, the DNA can be maintained either within the free virus or within a lysogenic cell, integrated into the bacterial chromosome or in some cases in the plasmid state. If a lambda vector contains potentially hazardous genes it is clearly advantageous for it to be un-

able to lysogenise bacteria; then only the virus (and the free DNA) needs to be 'contained'. A number of lambda mutations are known which reduce the frequency of lysogeny greater than 10<sup>6</sup>-fold. Luckily lambda has quite a narrow host range, probably being unable to infect most intestinal strains of *E. coli*. In addition, a number of mutations can be introduced to minimise its spread through sensitive bacterial populations. For example, Enquist *et al.* (page 596 of this issue of *Nature*) have introduced three nonsense mutations into a lambda vector to ensure that viable phage can only be produced in certain suppressor<sup>+</sup> bacteria, which it is hoped are not likely to be encountered by chance. Another approach is to propagate the vector on a non-modifying strain of bacteria so that the vector DNA will be restricted and degraded in any bacteria having a restriction system. Lambda derivatives containing these types of safety features are also being developed in Edinburgh, Paris, Stanford and Wisconsin.

Another possible hazard results from the fact that free vector DNA can be taken up and replicated by bacteria. Although this process is rather inefficient, the large-scale experiments necessary to obtain sufficient amounts of cloned DNA for some purposes pose a potential hazard. Consequently it is desirable to maximise the yield of vector per bacterial cell. Several thousand copies per cell of some plasmid vectors can be obtained under particular conditions, whilst lambda mutations that make the host lysis-defective are very useful since they increase the phage yield per cell up to 10 times and moreover the phage remain in the bacterial cell until artificially lysed making collection easier and safer. It is also an advantage for the vector to carry conditional-lethal mutations that prevent it replicating in an accidentally infected host under physiological conditions.

An alternative approach to safe genetic engineering is to develop vectors and host organisms that are neither pathogenic to animals and plants nor comprise part of their normal bacterial flora. The favoured organisms emerging from these studies are *Bacillus subtilis* and certain strains of *Pseudomonas*. These are normal soil inhabitants and grow well in defined media, but although a fair amount is known about the genetics and physiology of *B. subtilis*, little is known about the genetics of its potential vectors. By contrast, some *Pseudomonas* plasmids are quite well characterised, although they suffer from the disadvantage (for safety) of being transferable to and expressed in other strains and species. Also, some species of *Bacillus* and *Pseudomonas* are extremely patho-

genic and so again there is the necessity to construct 'safe' vectors whose accidental transfer throughout populations is eliminated. □

## Thyroglobulin mystery solved?

from J. R. Tata

FOR many, thyroglobulin is simply a convenient "marker" for analytical electrophoresis and gel filtration for calibration of molecular weights of proteins. For a few, thyroglobulin is a unique giant iodoglycoprotein (molecular weight of 660,000 for the native protein) made exclusively in the thyroid gland of all vertebrates. Its hydrolysis in the gland causes the release of the two thyroid hormones, thyroxine and tri-iodothyronine, which are themselves quite unusual iodoamino acids, known as iodothyronines. Although numerous proteins can be enzymically or chemically iodinated, it is only in the confines of the thyroglobulin molecule that a few privileged mono- and di-iodotyrosyl residues can couple to form the hormonally active iodothyronines covalently retained within the large polypeptide (Edelhoch, *Rec Progr. Horm. Res.*, **21**, 1; 1965). Because of the uniqueness of its composition and structure, and the clinical and physiological importance of thyroid hormones, the thyroglobulin molecule has been intensively investigated in the past 40 years. A consequence of this activity has been the controversy that has been generated in the past decade over its subunit structure and heterogeneity. The number of subunits proposed has varied from 2 to 10 with molecular weights ranging from 25,000 to 200,000. Two recent reports now seem to put an end to this controversy.

The first report is based on some elegant work by Gilbert Vassart and his colleagues in Brussels (Vassart *et al.* *Proc. natn. Acad. Sci. U.S.A.*, **72**, 3839; 1975). It exploits the technique of translating heterologous messenger RNAs in *Xenopus* oocytes, first developed in Gurdon's laboratory (Lane *et al.*, *J. molec. Biol.*, **61**, 73; 1971). The Belgian group had earlier immunologically characterised a 33S RNA extracted from membrane-bound polysomes from ox thyroids as mRNA coding for thyroglobulin (Vassart *et al.*, *Eur. J. Biochem.*, **55**, 5; 1975). What they have now done is to demonstrate by sucrose density gradient centrifugation and gel filtration under denaturing conditions that a "19S" non-iodinated thyroglobulin (corresponding to a protein of 600,000 molecular weight) was



the major immunoreactive product of translation of 33S mRNA in *Xenopus* oocytes. The identity of this peptide with thyroglobulin was confirmed by tryptic peptide analysis which led Vassart's group to propose that thyroglobulin is a dimer of two equal subunits of 300,000 daltons. Evidence obtained from more conventional techniques for a similar large subunit of thyroglobulin also comes from a recent report from Edelhoch's laboratory at the National Institutes of Health (Haerberli *et al.*, *J. biol. Chem.*, **250**, 7294; 1975). Ten years ago Harold Edelhoch proposed that thyroglobulin was composed of four smaller subunits. But the new work from the NIH laboratory, on the basis of equilibrium density centrifugation and gel filtration in 6M guanidinium HCl of guinea pig thyroglobulin, definitely establishes the presence of a subunit of about 300,000 daltons. Two smaller subunits of molecular weights of about 210,000 and 110,000 were also detected, but all three had the same amino acid composition as whole thyroglobulin, the only major difference between them being their different iodine contents. A major reason for the earlier observations of a variety of small subunits, and the failure to detect the large one of 300,000 daltons, may lie in the extreme susceptibility of thyroglobulin to both substrate-specific and nonspecific proteases, with which the thyroid gland is particularly well endowed.

There are some interesting points emerging from the Belgian work on microinjection of *Xenopus* oocytes. Earlier experience with the translation of several foreign RNAs in frog oocytes had shown that oocytes are capable of carrying a variety of post-translational modifications of the newly synthesised polypeptide (Lane and Knowland, *The Biochemistry of Animal Development*, **3**, 145; 1975). The recovery of non-iodinated thyroglobulin is the first evidence of a failure of the oocyte to carry out a post-translational modification of a polypeptide synthesised on a vertebrate mRNA. The recovery from the oocyte of such a large sized subunit of thyroglobulin reveals a striking protection against proteolysis of the polypeptide synthesised on the injected mRNA template, especially as the *Xenopus* oocyte is known to degrade certain proteins injected into it (Dehn and Wallace, *J. Cell Biol.*, **58**, 721; 1973). What is not yet known is whether or not the primary translational product in the oocyte is a "pro-thyroglobulin", or even a "pre-pro-thyroglobulin", in line with the increasing evidence that most, if not all, secretory proteins may be synthesised as larger precursors and then specifically cleaved in the membrane of endoplasmic reticulum. In this context,

it would be interesting to find out if thyroglobulin mRNA is translated on membrane-bound ribosomes of the oocytes as it is in the thyroid cell. It would also be interesting to determine whether the oocyte carries out the post-translational glycosylation of thyroglobulin, especially as this protein is unusual in its carbohydrate composition. Whatever mysteries post-transla-

tional modifications may hold, the work from the Belgian and American laboratories seems to have solved that of the subunit structure of thyroglobulin. The work by the Belgian group is also the first in which the technique of microinjection of RNA into frog oocytes or eggs has been successfully applied to answering questions concerning protein structure. □

## The Great Barrier Reef

### Geomorphology . . .

from George E. Farrow

At a meeting in London on January 28-29, David Stoddart (University of Cambridge) and Sir Maurice Yonge added the results of the 1973 Great Barrier Reef Expedition to the illustrious series of southern zone expeditions with which the Royal Society has long been associated.

COMPARED with other recent Royal Society expeditions such as the much longer Aldabra Expedition of 1967/8 with its vital 'natural history' emphasis, or with the Solomon Islands Expedition of 1965, there was a singular dearth of faunistic papers—in fact only one out of the 19 presented. The meeting was very much a discussion on the recent geological history of the reefs, particularly in relation to possible Holocene sea-level changes.

The 1973 Expedition was financed jointly by the Royal Society and the Universities of Queensland. It worked deliberately north of Cairns, in view of previous concentration on central and southern sectors of the Great Barrier, notably by the famous 1928/29 Expedition. Seventy islands were mapped, and a flora of 200 species collected from forty—a demonstrably Australian flora with many common pan-tropical plants missing.

The most striking achievement of the expedition must surely have been G. R. Orme's (University of Queensland) use of high resolution seismic profiles to demonstrate clearly the remnant nature of the present-day reefs. This lends strong support to Purdy's recent study of central American reefs which similarly showed that modern reefs inherit their structure from earlier, eroded reef limestones. Many submerged terraces were identified on the Barrier, those at -45 m and -109 m being strongly developed. The latter was associated with a buried valley system, overlying which a thick fluvial regressive sequence seems

likely.

More than 80 radiocarbon dates have so far been obtained from material collected during the expedition, dating being carried out at the Australian National University, Canberra. Rationalisation of the dates in terms of a 'Fairbridge-type' sea-level curve proved impossible, however, because of difficulties in establishing datum levels for both the present and the past. Uncertainty about the eustatic *versus* cyclonic significance of reef island terraces and ramparts made it clear that a critical reassessment is needed of many existing dates used for sea-level reconstructions in the literature.

Though it is premature to attach significance to the dates, several interesting clusters appeared. Microatolls, some with elevations greater than the highest modern 'ponded' examples, commonly date at around 5,500 BP, the bigger cays around 3,500 BP, with two groups of beach rock at 3,000 BP and 2,000 to 1,500 BP. Even the most mobile sand cays give dates which indicate that the sediment is at least 2,000 yr old. Indeed, one came away from the discussion feeling decidedly palimpsest.

A disappointing aspect of the age dating was that suspected pre-Holocene material recovered from a 30-m hole drilled on Bewick Island proved too recrystallised for Uranium Series age determination.

D. R. Stoddart presented clear evidence, by the remapping of Low Isles (so elegantly mapped by Spender in 1928/9), of the major changes that can overtake islands in a very few years. Consternation overtook several members of the 1928/29 Expedition on hearing that the eroded foundations of their headquarters were mapped in 1973 as part of the intertidal zone! An evolution is to be recognised in the sequence of environments (and therefore in the sediments) that results from the diachronous leeward progres-

sion of belts of mangrove, themselves initially dependent on a degree of stability imparted by storm ramparts. But there is little to suggest that such an evolutionary sequence would be synchronous on neighbouring islands.

With the recent establishment of the Australian Institute of Marine Science at Townsville, a new phase of intensive long term study of ecological and sedimentary processes has become possible. This should go a long way towards rectifying our present ignorance of scale and rate factors operating in the overall barrier system.

In order to capitalise on the geophysical work of the expedition a series of boreholes at intervals across the shelf is needed to demonstrate the nature and sequence of the suspected major Holocene fluvial development overlying the -109-m terrace: until this is done the interpretation of seismic evidence must remain conjectural. Such a study would be well complemented by detailed examination of the aggradational sequences along the Queensland coast.

On a more local scale the expedition has demonstrated that the time is ripe for 'taking an island apart' by systematically drilling a series of holes through a single island like Bewick, with a view to reconstructing in three, rather than in two, dimensions the evolution of the modern reef geometry. Again, the seismic survey has suggested the potential rewards that such a drilling programme might bring in revealing exactly how the morphology of the earlier dissected reef limestone surface influences the subsequent growth and development of the modern reef system.

Over the next few years, therefore, we should see a new understanding emerge of the internal structure of the Great Barrier Reef, even perhaps including an appreciation of its foundation structure in relation to the plate-tectonic history of the western Pacific.

But there is surely another side to all this. Plate tectonics is all very well, but have we not forgotten the organisms—the very substance and most singular feature of the reefs? Have we yet begun to appreciate, for example, the immense potentiality that the 1,600 km length of reef offers for latitudinal studies of organic diversity? As it is, the basic fieldwork still remains to be done, to enable the spectrum of reef communities of the northern Barrier to be compared with those at Low Isles, let alone with other Indo-Pacific reefs. So we can confidently look forward to a resurgence of interest in the natural history of the Great Barrier, and the despatch of a further, faunistically orientated expedition. □

... and ecology

## Reef biogenesis

from Jean H. Weber

A symposium on coral reef biogenesis was organised by the Australian Institute of Marine Science (AIMS) on December 15–16, 1975 in Townsville, Queensland, Australia. The long term objective of the AIMS work on coral reef biogenesis is to develop a unifying model of the growth, development, and maintenance of the Great Barrier Reef, and the symposium was held to identify critical areas of research. Further details may be obtained from the Director, AIMS, Townsville, Queensland 4810.

M. LITTLER (University of California, Irvine) reviewed and evaluated the literature pertinent to the calcium-depositing habit in macroalgae, noting the current debate on the relative ecological importance of various kinds of calcifying organisms. Suggested research areas included partitioning pH changes into those due to calcification and those due to photosynthesis, by following changes in total alkalinity of the system by chemical methods. Studies of periodicity and stress effects on deposition rates were also recommended, as well as of grazing pressure on reef-building algae, inter and intra-specific competition, recruitment, and bioenergetics.

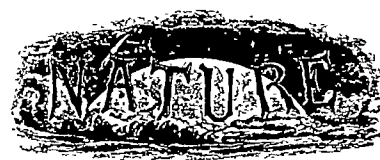
One vital group of reef algae are the symbiotic zooxanthellae, which make possible the survival of reef corals in a nutrient-poor environment. L. Muscatine (University of California, Los Angeles) evaluated the state of knowledge of the coral/zooxanthellar symbiosis. Some fundamental questions still remain unresolved (what, for example, is the quantitative contribution of zooxanthellae to maintenance respiration of a coral? what is the mechanism of nutrient retention and recycling? how does photosynthesis accelerate calcium deposition?).

Marine productivity begins with photosynthesis and the production of metabolic intermediates. A. Benson (Scripps Institution, San Diego) noted that the importance of lipids in the energy budget of a wide variety of reef organisms was first suggested by the recognition of wax esters, triglycerides, and phospholipids as components of mucus secreted by corals and other reef organisms. This mucus is consumed by various animals, including fishes and many commensals. Reef corals derive their stores of lipid by a yet undefined transfer process from their symbiotic zooxanthellae.

M. Doty (University of Hawaii) reviewed the essential functions of the serially appearing algal components of coral reefs: the planktonic (micro-), fleshy (meso-), and crustose (macro-morphic) constituents.

Coral reefs are unique among marine ecosystems in their ability to produce biogenic sedimentary materials and to retain these materials in the form of a wave-resistant structure. This ability to alter the environment is a function both of the rate of production and the nature of the material produced. The growth of reefs is mediated initially by an environmental "coarse-tuning", imposed by geological, meteorological and oceanographic variables. The reef system itself then acts as a "fine-tuning" device. S. Smith (Hawaii Institute of Marine Biology) examined the nature of CaCO<sub>3</sub> materials produced, rates of production, and the external factors which influence and are in turn influenced by the growth of coral reefs.

D. Stoddart (University of Cam-



## A hundred years ago

MOST of the natives who come into intimate contact with the Russians at the present time, we are informed, profess Christianity. That many heathen customs still, however, cleave to them is shown by the following incident. At a "simovie" where we landed for some hours on Sept. 16, we as usual came upon a burying-place in the wood near the dwelling-houses. The corpses were laid in large coffins above ground, with a cross in nearly every case raised over them. At one of the graves there was a consecrated picture fixed to the cross, which must be considered an additional proof that a Christian reposed in the coffin. Notwithstanding this, several garments, which had belonged to the deceased, were found hanging on a bush near the grave, together with a bundle containing food, principally dried fish. At the graves of the richer natives we are informed that the survivors place, together with food, some rouble notes, in order that the departed may not be altogether destitute of ready money on his entrance into the other world. But that fine clothes are not considered any special recommendation with St. Peter was evidenced by the exceedingly shabby, tattered, and patched condition of the garments hung up at the grave in question.

from *Nature*, 13, February 17, 313; 1876.

bridge) outlined the geological aspects of reef biogenesis. External form has been used to infer both history and internal structure, without itself being precisely defined. To understand variation in time and space, reefs must be considered as volumes or three-dimensional shapes, as opposed to the commonly-used profile diagram, which is well-adapted to describe zonation but unable to demonstrate spatial variability. New advances in describing reef form have included the use of nearest-neighbour techniques for studying patch-reef distribution, the use of spectral analysis of bottom roughness to describe groove-and-spur formations, the application of the hypsometric integral to describe lagoon basins, and the development of various indices of topographic diversity to describe reef communities. Such quantitative morphometric techniques can be applied on different scales, from entire reef complexes to individual coralla. Until three-dimensional reef forms have been described more precisely in this way, the heavy theoretical loading given to highly simplified reef descriptions must be misleading. □

## Spatial contrast vision

from a Correspondent

A Workshop on Spatial Contrast Vision was held in Amsterdam on 9–14 January 1975. It was sponsored by the Commission for Biophysics and Biochemistry of the Netherlands Royal Society of Arts and Sciences.

VISUAL scientists tend to be divided into two camps on the question of how patterns are perceived. One view is that visual information is processed by one or more frequency-selective channels; a strong statement of this theory is that the visual system performs a two-dimensional Fourier analysis. The opposing camp holds that the fundamental building-blocks are the responses of single neurones, the receptive fields of which are sensitive only to specific features of the stimulus; the strong statement is that more central neurones are increasingly selective, and that an individual percept depends ultimately on activity in a single high-order neurone.

This dichotomy could be found in much of the discussion at the Amsterdam meeting. L. H. Van der Tweel (University of Amsterdam) criticised misapplications of Fourier theory: in particular, the tendency to

ignore the question of how phase information is preserved in vision. It was later shown by Ü. Tulunay-Keesey (University of Wisconsin) that phase information can be lost by frequency-selective channels, because equal adaptive effects are found when adapting and test gratings are present in phase or out of phase. This finding raises the question of how spatial channels can ever specify the position of an object.

J. Nachmias (University of Pennsylvania) considered whether vision could be mediated by a single channel, the sensitivity of which varied with the spatial frequency of the stimulus. He concluded that such a model cannot account for known data; a multiple-channel theory is needed. This problem was discussed further by J. G. Robson (University of Cambridge), who described problems in the interpretation of human psychophysical data in terms of the properties of neural receptive fields. The classic concept of a receptive field is of a centre, within which all like stimuli elicit similar responses, and a surround, within which the same stimuli have an opposing effect. New evidence that this is an oversimplification was presented by P. O. Bishop (Australian National University), who described local differences of function within receptive fields. Sensitivity to the direction of stimulus movement can be found in small areas of the receptive field, and cells that exhibit directional sensitivity within the centres of their fields often have non-selective surrounds.

The convention of classifying retinal ganglion cells as X and Y cells was proposed by Enroth-Cugell and Robson in 1966, on the basis that X cells perform a linear summation of light within their receptive fields. The distinction has proved to be useful: neurones with X and Y characteristics have now been found in other parts of the visual system, and they differ in several characteristics. C. Enroth-Cugell (Northwestern University) reported that X and Y cells have different profiles of spatial sensitivity, and may have different neurochemical transmitters. R. M. Shapley (Rockefeller University) showed that only X cells respond linearly to temporal modulation of light. The central projections of X and Y cells differ, as shown by K.-P. Hoffman (Gutenberg University, Mainz). The X cells project only to area 17 of the visual cortex, whereas Y cells project to areas 17 and 18. Hoffman also described a third major category of visual neurones, the W cells, whose only unifying characteristic is their slow conduction velocity.

The ability of many neurones to respond to a limited range of spatial

frequencies was examined by R. L. DeValois (University of California) and L. Maffei (Laboratorio di Neurofisiologia, Pisa). In general, tuning curves become more sharply peaked as one ascends in the visual system. Considerable interest (and perhaps some scepticism) greeted Maffei's conclusion that all neurones that are located in the same column of visual cortex (that is, in a line perpendicular to the surface) tend to respond most vigorously to gratings that have the same orientation, but their preferred spatial frequency changes in a regular pattern as the recording electrode is advanced. Conversely, cells in hypercolumns (tangential to the surface) have different preferred orientations, but all respond to the same spatial frequencies.

Most of these findings emphasise spatial interactions of an inhibitory nature. A. Fiorentini (Laboratorio di Neurofisiologia, Pisa), however presented compelling data that both facilitatory and inhibitory interactions can be found in human vision.

During a 'critical period' in early life, the visual system of many animals is labile and its function can be modified substantially by depriving or distorting the visual input. This phenomenon has been studied extensively because of its implications in the treatment of human amblyopia. C. B. Blakemore (University of Cambridge) described recent experiments in which occlusion of one eye resulted in a selective loss of afferent fibres to the visual cortex from those parts of the lateral geniculate nucleus to which the occluded eye projects. J. Atkinson and O. J. Braddick (University of Cambridge) reported that the contrast vision of human infants can be tested as early as 1 month, and that a rapid improvement in sensitivity occurs during the second month of life. This raised the question of the age at which the critical period begins in man. Bishop stated that the literature suggests 5 months, but Atkinson and R. A. Crone (University of Amsterdam) argued for an earlier onset.

A wide gap still exists between experimental data and an understanding of visual perception. This was emphasised by E. H. Land (Polaroid Corporation), who gave a dramatic demonstration that knowledge of the properties of a discrete physical stimulus is not sufficient to predict its appearance, and by N. S. Sutherland (University of Sussex), who attacked the notion that the central nervous system analyses visual information in a passive manner. In so doing, Sutherland seems to have questioned the meaningfulness of the two popular models of contrast vision that are stated at the beginning of this report.

# articles

## Hazards of plutonium with special reference to the skeleton

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*In the past attempts have been made to deduce plutonium toxicity in man from studies based on animal experimentation. An alternative method is to use the comparative dosimetry of plutonium and radium in man, with results broadly in agreement with maximum permissible levels set by the International Commission on Radiological Protection.*

SEABORG recognised as early as 1944 that "the physiological hazards of working with plutonium and its compounds may be very great"<sup>1</sup>. Subsequent experimental work on the biological behaviour of plutonium has confirmed Seaborg's fear about his new element and has shown that it is more carcinogenic than radium. As increasing quantities of plutonium will be produced in connection with nuclear energy programmes, it is important to have some estimate of the risks involved, namely the possible incidence of malignant cancers in individuals who have acquired a plutonium burden in the course of their work.

We present here some estimates of the risk of development of malignancies associated with the skeleton, based on a dosimetric comparison with the incidence of radium-induced cancer in man, about which considerable evidence exists<sup>2,3</sup>. Both <sup>226</sup>Ra and <sup>239</sup>Pu emit  $\alpha$  particles with not very different energies and both are deposited in bone; <sup>226</sup>Ra is deposited throughout the volume of mineral bone whereas <sup>239</sup>Pu is deposited on bone surfaces, with some distributed in bone marrow. Our calculations, however, take these differences into account since they are based, as UNSCEAR<sup>4</sup> recommends, on the known cells at risk and the determination of the physical dose these cells receive. Similar dose calculations cannot yet be made for the other major sites of plutonium retention, the lung and the liver, because the cells at risk are not known with the same certainty.

### Cells at risk in the skeleton

The cells at risk in the case of the skeleton are the progenitor osteogenic cells on bone surfaces, all the progenitor cell types found in the marrow and cells in the epithelium closely applied to bone in the sinuses of the skull<sup>5,6</sup>. The following brief review illustrates the evidence available that malignancies have been found in all these tissues, in animals and man, when irradiated by  $\alpha$  particles. Of the tumours listed, osteosarcomas are by far the most numerous

The data on <sup>239</sup>Pu refer to experimental animals with known

plutonium burdens. In 75 dogs injected with <sup>239</sup>Pu (ref. 7), 55 osteogenic sarcomas were recorded, compared with one case of leukaemia. There were no osteosarcomas in the controls but one leukaemia occurred.

In rats studied by Benstead *et al.*<sup>8</sup> 45 osteosarcoma were recorded against four cases of myeloid leukaemia. Russian workers<sup>9</sup> comment on a high incidence of tumours of the blood-forming organs in rats but data on osteosarcoma are not given. In extremely careful studies in mice, Loutit (personal communication) has recorded generalised lymphomas and local lymphomas as well as osteosarcomas and angiosarcomas. The mouse studies of Finkel and Biskis<sup>10</sup> show a high incidence of both osteosarcomas and reticular tumours, but are difficult to assess because of the large number of reticular tumours in the controls. One carcinoma of the sinuses is recorded by Jee<sup>7</sup> in a beagle dog whereas none were seen in controls.

No malignancies attributable to plutonium have been recorded in man, although in some cases the body burdens have been as high as 0.42  $\mu$ Ci (ref. 11). Tumours have occurred however, in a large group of persons (1,346 in all) exposed to radium, as either <sup>226</sup>Ra or <sup>228</sup>Ra or a mixture of the two. For this population information is available on radium body burden, calculated radiation dose received and subsequent pathology<sup>2,3,12,13</sup>. The total number of osteosarcomas in the radium study when last reported was 51; there were 22 cancers of the sinuses<sup>3,13</sup>.

Some patients had both types of tumour. Finkel and his colleagues<sup>2</sup> include in their record two cases of myeloid leukaemia and three other less well defined blood dyscrasias. When these figures were taken from the recent analysis of the radium cases, some of the early patients described by Martland<sup>14</sup> were not included by Rowland *et al.*<sup>3</sup>. In addition to osteogenic sarcoma, these early cases had atypical dysplasia of the marrow associated with severe anaemia. Their body burdens of radium were extremely high. Loutit<sup>15</sup> has suggested that they should be regarded as samples of malignant transformation of marrow cells. The risk of leukaemia from a plutonium burden is possibly greater than that from a radium burden as plutonium is retained in marrow as well as in the bone itself<sup>16-21</sup>. An ICRP publication<sup>22</sup> therefore suggested in 1972 that the skeleton probably should be considered as two distinct organs, one comprising the cortical and trabecular bone and the other the bone marrow. It may be noted that the time taken for leukaemia to develop is likely to be longer than in the case of external radiation since plutonium, entering the body by inhalation or tissue wounding, will translocate slowly and continuously from lungs, lymph nodes, wound site, or initial liver deposits. The marrow dose will build up slowly with time<sup>21</sup>.



## Animal work and toxicity estimates

Attempts have been made to relate the toxicity of a known body burden in dogs injected with different radionuclides to known body burdens of radium in man using the formula

$$\frac{\text{dose from X in animal}}{\text{dose from } ^{226}\text{Ra in animal}} = \frac{\text{dose from X in man}}{\text{dose from } ^{226}\text{Ra in man}}$$

where the doses refer to some given level of toxicity.

The doses for dogs and humans are based on an average distribution of both radionuclides in bone, that is on the average dose to mineral bone. The UNSCEAR report<sup>23</sup> makes a general comment on such types of comparison between radium data for man and animal data: "this question carries the assumption that the ratio of the absorbed doses which produce different effects for different radionuclides will be the same in man and the experimental animal, independent of promoting host factors which might act differentially, an assumption that at present remains unproved". The ratio of the doses to the cells at risk for <sup>239</sup>Pu and <sup>226</sup>Ra are not necessarily the same as the ratio of the absorbed doses to bone. Apart from the fact, pointed out by UNSCEAR<sup>23</sup>, that it is unsatisfactory to base toxicity estimates on average dose to mineral bone when it is dose to sensitive tissues that is important, there are other difficulties involved in this comparison of data for dogs and humans. First, Spiers<sup>24</sup> has shown by direct measurement of both dog and human bone that considerable structural differences in the two species affect the dose to the sensitive tissues in marrow and on bone surface and are not taken into account by the average dose to mineral bone.

Table 1 Calculated dose rates

Radionuclide	Mean dose rates (rad yr <sup>-1</sup> ) for 1 µCi skeletal content*			
	Trabecular marrow	Trabecular endosteum	Cortical endosteum	Air-sinus epithelium
<sup>226</sup> Ra + retained daughter elements	1.6 (1.5)†	19 (18)†	35	275‡
<sup>239</sup> Pu	16.9	129	193	193‡

\* Bone data assumed: skeletal mass = 5 kg, total endosteal area = 16 m<sup>2</sup>.

† Deduced from data (plane slab calculation) by Charlton and Cormack<sup>31</sup>.

‡ Estimate only.

Second, since plutonium is a surface seeker, the rate at which resorption and apposition occurs will affect the dose received by sensitive tissues. There is no reason to suppose that 'turnover rate' is the same in man and dog and it will certainly not be the same in young dogs and adult man as is accepted in the above formula. Marshall and Lloyd<sup>25</sup> have indeed drawn attention to the fact that it is unwise to compare toxicity in young dogs with that in adult man since the rate of bone turnover, that is both removal of plutonium-containing bone and its burial by new bone formation, may be different. As a result of their calculations of different remodelling times in young dogs and adult man, Marshall and Lloyd conclude that in the case of monomeric plutonium the relative biological effectiveness of plutonium compared with radium (RBE) in man is about three times that in dogs.

The differences between the skeletons of young dogs and adult humans are again emphasised by a study<sup>26</sup> of the relative carcinogenicity of <sup>226</sup>Ra and <sup>228</sup>Ra in man and a comparison with the results for the Utah dogs<sup>7</sup>. In the case of the Utah dogs, <sup>228</sup>Ra was more carcinogenic than <sup>226</sup>Ra with an RBE of 2.5. Rowland *et al.*<sup>26</sup> concluded, however, that in man there was no difference in the toxicity of the two isotopes. As they say, these observations make it difficult to accept that toxicity ratios observed in beagles can be used directly to predict toxicity ratios

in man. Although examination of the data they give for cumulative dose levels above 1,000 rad does not altogether substantiate their claim that there is no statistical difference between the toxicity of <sup>228</sup>Ra and <sup>226</sup>Ra in man, it seems probable that the comparison of the data for dogs and humans gives too high an RBE for <sup>228</sup>Ra relative to <sup>226</sup>Ra in humans. On theoretical grounds ICRP<sup>27</sup> has put the maximum permissible body burden (MPBB) for <sup>228</sup>Ra lower than that for <sup>226</sup>Ra.

Since considerable reservations must be made in interpreting simple translation to man of toxicity data obtained with dogs, it is worthwhile attempting to estimate the toxicity of <sup>239</sup>Pu by a method that uses the data for <sup>226</sup>Ra in man coupled with new calculation of dose to the relevant tissues.

## Dose rates calculated from skeletally deposited radionuclides

A method of calculating the radiation dose to tissues in bone from skeletally deposited radionuclides has been devised that takes into account the physical dimensions of the trabeculae and marrow spaces in trabecular bone<sup>24, 28, 29</sup>. It is based on the principle that particles cross the small marrow cavities in approximately straight lines with varying lengths of path. A complete description of all possible path lengths through the marrow spaces and through the trabeculae makes it possible to calculate the fraction of energy deposited, and hence the dose, in the marrow spaces by particles arising in the trabeculae. This is done by a Monte Carlo method operating in probability distributions of random path lengths measured by a specially-designed bone-scanning microscope<sup>30</sup>. The dose is averaged over the whole distribution of path lengths to give a mean value for a given bone, and sufficient bone specimens are analysed for a mean dose for the whole skeleton to be obtained. Mean dose rates have been calculated for a skeletal content of 1 µCi of a given radionuclide for the following tissues: (1) bone marrow in trabecular bone (*D<sub>M</sub>*); (2) endosteal tissue, 10 µm thick, on trabecular bone surfaces (*D<sub>S</sub>*); (3) endosteal tissue on the surfaces of cavities in cortical bone (*D'<sub>S</sub>*), and (4) epithelial tissue adjacent to bone in the air sinuses (*D<sub>AS</sub>*).

Table 2 Calculated risks

Tissue	Late effect	Risk coefficient: cases per 10 <sup>3</sup> per yr for 1 rad yr <sup>-1</sup>
Trabecular marrow	Leukaemia	<i>r<sub>M</sub></i> = 0.101
Trabecular endosteal surfaces	Osteosarcoma	<i>r<sub>OS</sub></i> = 0.079
Cortical endosteal surfaces	Osteosarcoma	<i>r'<sub>OS</sub></i> = 0.016
Air-sinus epithelium	Carcinoma	<i>r<sub>AS</sub></i> = 0.003

The method of calculation, developed initially for β emitters, assumes that the radionuclide is deposited uniformly throughout the volume of the mineral bone. It can be applied equally to a "volume-seeking" α emitter such as <sup>226</sup>Ra and results for this radionuclide are given in Table 1. Where comparisons can be made, the dose rates agree well with those derived from data by Charlton and Cormack<sup>31</sup> for the dose near a plane slab of bone containing <sup>226</sup>Ra (Table 1).

In the case of those radionuclides, such as <sup>239</sup>Pu, that are deposited on bone surfaces the Monte Carlo program must be modified to consider particles as originating only at the endosteal points of any given trabecular path. A further factor must be introduced to allow for the contribution to the dose from the trabecular surface immediately adjacent to the cavity concerned. The details of the calculation for "surface-seekers" will be published later, but approximate dose rates for a skeletal content of 1 µCi <sup>239</sup>Pu are given in Table 1. For the purpose of this article it has been assumed that 10% of the skeletal content could be deposited in bone marrow<sup>21</sup>, with the remainder distributed uniformly over the endosteal surfaces of the

skeleton. The dose rates calculated for  $^{239}\text{Pu}$  agree reasonably well with those based on a thin plane source. Thus the dose rate for the trabecular endosteum is about 10% less than that calculated for a thin plane<sup>32</sup>. It is about 25% less than the value deduced from the data of Marshall *et al.*<sup>33</sup> but in this case uniform energy deposition along the particle track was assumed. Dose rates near trabecular surfaces may be expected to be somewhat lower than for a thin plane source because the trabecular surfaces are not flat and the marrow spaces are generally too large for appreciable cross contributions to the dose from other trabecular surfaces. No allowance has been made for possible burial of the Pu by bone growth because insufficient evidence is available; the dose rates in Table 1 are therefore likely to be maximum estimates. In the case of  $^{238}\text{Pu}$ , the  $\alpha$ -particle energy and the consequent dosimetry are almost the same as for  $^{239}\text{Pu}$ , though it must be remembered that because of its high specific activity  $^{238}\text{Pu}$  does not necessarily behave biologically like  $^{239}\text{Pu}$ .

### Calculation of total risk to bone

The principle of the calculation<sup>34</sup> depends on a knowledge of the risk coefficients for the relevant late effects (leukaemia, bone sarcoma and air-sinus carcinoma) when bone tissues are irradiated by  $\alpha$  particles. The coefficients are expressed as the equilibrium risk rates, namely, the number of cases per 1,000 persons per year when a dose rate of 1 rad yr<sup>-1</sup> is maintained for a sufficiently long time. The 'equilibration' time depends on the latency period of tumour appearance, and for irradiated bone this is likely to be long—bone tumours have occurred from 10 to 30 yr after irradiation.

The total risk rate,  $R_1$ , for a skeletal content of 1  $\mu\text{Ci}$  of a given radionuclide can be stated as

$$R_1 = r_M D_M + r_{OS} D_S + r'_{OS} D'_S + r_{AS} D_{AS} \quad (1)$$

where  $r_M$  is the risk coefficient for leukaemia per unit dose rate and  $D_M$  is the dose rate to bone marrow per  $\mu\text{Ci}$  skeletal content;  $r_{OS} D_S$  refers similarly to bone sarcoma in endosteal tissues in trabecular bone,  $r'_{OS} D'_S$  to bone sarcoma in cortical bone and  $r_{AS} D_{AS}$  to air-sinus carcinoma. Clearly other risks, if any, could be added but these particular ones will be considered in respect of the  $\alpha$  irradiation of bone.

and the total risk rates to bone are as given in Table 3 for  $^{226}\text{Ra}$  and  $^{239}\text{Pu}$ . The risk estimates for plutonium are made on the almost axiomatic assumption that the effect of unit  $\alpha$ -particle dose, delivered under the same conditions to the target cells concerned, does not depend on the radionuclide from which the  $\alpha$  particles originate (apart from possible minor changes in RBE with  $\alpha$ -particle energy).

The data in Table 3 can be used to derive a quantity,  $q$  ( $\mu\text{Ci}$ ), which we can define as the skeletal content which results in some stated maximum risk rate  $R_{MPL}$ ; the value of  $q$  is then obtained from the relationship

$$q R_1 = R_{MPL} \quad (2)$$

If bone only is irradiated by the bone-seeking radionuclide, the value of  $R_{MPL}$  could be taken, for example, as the risk rate associated with the limit 5 rad yr<sup>-1</sup>, the maximum permissible dose rate set by ICRP for radiation workers. This value can be deduced from the conclusions of the UNSCEAR report<sup>36</sup> as about 0.5 cases per 1,000 per yr, and  $q$  is then given by

$$q = 0.5/R_1 \quad (3)$$

Values of  $q$  are listed in the penultimate column of Table 3 and the present ICRP values of the MPBB<sup>27</sup> in the last column. It may be noted that, in deriving  $q$ , linear dose-response relationships have been assumed on both sides of equation (2), also that the occurrence of air-sinus carcinoma in the radium series is almost certainly due to heavy  $\alpha$  irradiation resulting from trapped radon in the poorly ventilated sinus cavities, which does not happen with plutonium.

The ICRP figures are based on the MPBB of 0.1  $\mu\text{Ci}$  assigned to  $^{226}\text{Ra}$ , a value that has remained unchanged for some 30 yr. The limit 0.1  $\mu\text{Ci}$  was set by the clinical evidence of bone cancer that had occurred by the early 1940s in the comparatively large group of persons who had acquired a body content of radium. The values of the MPBB for other  $\alpha$  emitters are set by the effective energy deposited in the skeleton compared with that deposited by 0.1  $\mu\text{Ci}$   $^{226}\text{Ra}$  and the daughter elements in the  $^{226}\text{Ra}$  decay chain<sup>27</sup>. The ICRP values in Table 3 are based on a deposition of 99% of the body content of  $^{226}\text{Ra}$  and 90% of  $^{239}\text{Pu}$  in the skeleton<sup>27</sup>. Some comparison can therefore be

Table 3 Risk rates

Radionuclide	Risk rate: equilibrium no. cases per 10 <sup>3</sup> per yr for maintained skeletal content of 1 $\mu\text{Ci}$					$q$ ( $\mu\text{Ci}$ )	MPBB (ICRP) ( $\mu\text{Ci}$ )
	Leukaemia	Bone sarcoma		Carcinoma	Total		
		Trabecular	Cortical				
$^{226}\text{Ra}$	0.2	1.5	0.6	0.8	3.1	0.16	0.1
$^{239}\text{Pu}$	1.7	10.2	3.1	0.6	15.6	0.032	0.04

The values of the risk coefficients can be deduced from the data for the radium cases. In 777 cases reported by Rowland *et al.*<sup>3</sup> the mean dose to bone was 1,860 rad and the malignancies observed over 40 yr were: 51 cases of osteosarcoma and 20 cases of air-sinus carcinoma. In another analysis by Finkel *et al.*<sup>2</sup> covering many of the same cases there were several blood dyscrasias of which at least three were acceptable as myeloid leukaemias (case histories and histology seen by J. V.). For our purpose of risk calculation we have taken the perhaps arbitrary figures of four cases of leukaemia. Details of the derivation of the risk estimates for  $\alpha$  particles will be given elsewhere. The calculations are on a linear dose-response basis and allow for the power-law reduction of dose rate with time after acquisition of the radium<sup>36</sup>. The results are given in Table 2, as the equilibrium numbers of cases per 1,000 per yr for a maintained dose rate of 1 rad yr<sup>-1</sup>.

If the dose rates in Table 1 are multiplied by the corresponding risk coefficients, the risk rates to the various tissues in bone

made between the skeletal contents,  $q$ , and the MPBB values. The comparison can only be approximate because the ICRP recommendations<sup>27</sup> require that the MPBB for long-lived radionuclides, such as  $^{226}\text{Ra}$  and  $^{239}\text{Pu}$ , shall only be reached after 50 yr occupational exposure. In our calculations the values of  $q$  are the skeletal contents which, if maintained, will result in the stated risk rate by the end of an equilibration time of some 30 yr or more. Nevertheless, the general concordance between two very different approaches to determining a maximum permissible skeletal content is such that the MPBB for  $^{226}\text{Ra}$  still seems valid and that for  $^{239}\text{Pu}$  is not in need of major revision in respect of bone. If, however, more recent ICRP data<sup>22</sup> on the distribution of  $^{239}\text{Pu}$  in the body are considered (45% in bone and 45% in liver), and the liver risk can be assessed adequately, some revision of the MPBB for  $^{239}\text{Pu}$  may be necessary.

The data in Table 3 can also be used to calculate the expected number of cases if a skeletal burden of 0.04  $\mu\text{Ci}$   $^{239}\text{Pu}$  were

maintained for about 50 yr; the expectation would be about two cases of leukaemia and 13 cases of bone sarcoma per 1,000 persons so exposed. Such estimates are necessarily tentative in view of the assumptions involved; they may be too high because the dose-response relationship so far reported for  $^{226}\text{Ra}$  is non-linear and a more detailed analysis (as that by Rowland *et al.*<sup>3</sup>), might lead to much lower estimates.

## Implications and conclusions

Because, as we have shown, it is difficult to translate animal toxicity data directly to man, there is an advantage in estimating plutonium toxicity on  $\alpha$ -particle dosimetry in human bone and data on cancer induction in man by  $^{226}\text{Ra}$ . This has involved some simplifications of the very complex radiobiological problems involved. For example, we have followed the ICRP and UNSCEAR in assuming a linear dose-response relationship. Other relationships are possible and a detailed theoretical study of the consequences of adopting different dose-response formulae has been made by Mayneord and Clarke<sup>37</sup>. Any formula adopted would have to be applied to both sides of equation (2). Further, we have assumed uniform volume deposition of  $^{226}\text{Ra}$  and uniform surface deposition of  $^{239}\text{Pu}$  in the skeleton. If a linear dose-response relationship holds then non-uniformity does not affect the expectation of cancer induction; there is indeed some evidence<sup>38</sup> from the induction of bone sarcoma in mice by  $^{45}\text{Ca}$ ,  $^{90}\text{Sr}$  and  $^{226}\text{Ra}$  that tumour numbers follow the average bone dose rather than the dose in the "hot spots". It should also be noted that in the case of inhaled plutonium, and with wound contamination, the plutonium at the local site will change with time and generally the bone content and the bone dose rate will increase with time. Also the risk to cells in the marrow will arise in part from plutonium that has translocated from elsewhere and this, as in the case of Thorotrast, takes time to reach an effective level<sup>39,40</sup>.

In making our analysis we have not considered the 'hot particle' problem which is as yet incompletely resolved and which is especially important in relation to cancer risk in the lung<sup>41-44</sup>. We have some comments to make, however, which seem to be relevant in respect of the risk when only a few cells in any one organ are irradiated. Recently, Mayneord and Clarke have extended their studies of point sources of  $\beta$  particles<sup>36</sup> to the case of point sources of  $^{239}\text{Pu}$  (ref. 45), and found that a peak probability of malignant change,  $1.5 \times 10^{-10}$  per hot particle, occurs at an integrated particle activity of  $0.3 \mu\text{Ci s}$ . We find we can deduce a risk of the same order of magnitude from data on the occurrence of osteosarcoma in the long bones of the radium cases. Measurements of trabecular surface areas by Spiers *et al.* (to be published later) have shown that the incidence of osteosarcoma in segments of the long bones is proportional to trabecular surface area with an incidence over a period of some 40-50 yr, of  $2.4 \times 10^{-6}$  per  $\text{cm}^2$  of trabecular surface. Because the osteosarcoma cases represent various radium burdens, this correlation suggests a worst-case situation in which, over long periods of exposure, the endosteal surface doses approach a carcinogenic level and the outcome is proportional to the area, that is, to the number of endosteal cells put at risk. If we were to make the admittedly large extrapolation to a shell of cells surrounding a  $^{239}\text{Pu}$  hot particle of radius equal to the  $\alpha$ -particle range (35  $\mu\text{m}$  in tissue) we would get a risk of malignant change for the area concerned ( $1.6 \times 10^{-4} \text{ cm}^2$ ) of about  $4 \times 10^{-10}$  per particle.

If we accept the risk estimate of Mayneord and Clarke of  $1.5 \times 10^{-10}$  per particle and take an average lung residence time of 2 yr for insoluble particles, the particle activity for peak probability is  $0.5 \times 10^{-2} \text{ pCi}$  and the number of hot particles for one maximum permissible lung burden (MPLB) of 16,000 pCi is  $3.2 \times 10^6$ . The expectation of malignant change for one MPLB would then be  $5 \times 10^{-4}$  spread over a long period of years. If one MPLB were received annually over 50 yr the total expectation of lung cancer would be about 12 cases per 1,000, not very different from the total risk to bone and bone marrow given earlier in this article. Reservations must be made in regard

to all such calculations because no account can be taken at present of the effects of particle movement.

## Summary

Since our calculations of "maximum risk" skeletal contents for the radionuclides  $^{226}\text{Ra}$  and  $^{239}\text{Pu}$  accord well with values of the MPBBs arrived at by the ICRP in 1959 on other grounds, we conclude that the MPBB of  $0.04 \mu\text{Ci}$  for  $^{239}\text{Pu}$ , then agreed, is not in need of major revision in respect of bone. A maintained skeletal burden of  $^{239}\text{Pu}$  of  $0.04 \mu\text{Ci}$  would lead, on our calculations, to possibly two cases of leukaemia and 13 cases of bone sarcoma per 1,000 persons over about 50 yr.

Carcinoma of the air sinuses would present a very much smaller risk than bone cancer. The risk to the lung cannot be so readily evaluated because, among other uncertainties, the cells at risk and the possible movement of the sources of radiation are little understood.

The fact, however, that recent studies by Mayneord and Clarke lead to risk estimates for the lung that are of the same order as those we find for bone suggests to us that, as far as we know, the maximum permissible lung burden set by the ICRP can also reasonably be accepted.

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# Quantitative assessment of carcinogenic risks associated with 'hot particles'

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*We have examined the hot particle problem in terms of the number of irradiated cells and the doses to which they are subjected, assuming both linear and nonlinear dose-response functions typical of those reported in the radiobiological literature. We assess the risk associated with point sources of  $\alpha$ ,  $\beta$  and  $\gamma$  activity compared with uniform irradiation, as well as the effects of fractionation of a single particle, and the change in risk if the particle moves. Finally, although the biological data are insufficient for firm estimates, we have tentatively attributed absolute carcinogenic risks in these various situations.*

THE recommendations of the International Commission on Radiological Protection assume that there is no threshold in the biological response to radiation, and that effects increase linearly with dose<sup>1</sup>. As a consequence, the average dose to an organ is a measure of carcinogenic risk, and concentration of the same energy into smaller volumes, which thereby locally sustain higher doses, leads theoretically to an identical probability of overall biological effect. The assumption of any form of dose-response relationship other than linear implies that spatial distribution of energy deposition may be important.

A special case of great theoretical and practical importance is the point source of  $\alpha$  or  $\beta$  activity, which subjects only a small fraction of the cells of an organ to very large doses. This 'hot particle' problem has become the subject of considerable controversy since Tamplin and Cochran<sup>2</sup> concluded that the associated tumorigenic risk is many orders of magnitude greater than that estimated from mean organ dose on the hypothesis of linearity. More recently, Lovins and Patterson<sup>3</sup> have questioned whether the present protection levels for  $\alpha$  emitters are adequate. The biological evidence concerning this alleged increased risk has been reviewed by Bair, Richmond and Wachholz<sup>4</sup> and also by the UK Medical Research Council<sup>5</sup>, both reports concluding that "there is at present no evidence to suggest that irradiation of the lung by particles of plutonium is likely to be markedly more carcinogenic than when the same activity is uniformly distributed".

## Theoretical model

Our fundamental assumption<sup>6</sup> is that the biological response of a single cell (that is, the probability of a relevant biological effect) is some function  $\phi(p_c D)$  of dose  $D$ , where  $p_c$  is the probability of malignant disease per cell per unit dose. The value of  $p_c$  has often been assumed constant, but is, in reality a

function of position, since the probability of effective emergence of a cell after 'transformation' may depend on local physiological and histological conditions, oxygen tension, cell contacts and the like. Indeed  $p_c$  is a function of many biological and physical variables, and for a given system may be periodic, varying during the cell cycle. Assuming, however, an overall mean value of  $p_c$ , and that the dose is spatially represented by  $D = f(x, y, z)$ , the overall expectation ( $\epsilon$ ) of malignancy in an organ or tissue may be written

$$\epsilon = \int n \phi(p_c f(x, y, z)) dV \quad (1)$$

where  $n$  is the number of cells per unit mass of tissue liable to undergo the effect.  $n$  will also probably be a complicated function of position and may also be a function of time.

The response,  $\phi(p_c D)$ , may take a variety of forms, but those of particular interest to us are the experimentally observed carcinogenic dose-response relationships. These often behave as power laws of dose at low doses, rise to a maximum in the region of a few hundred to a few thousand rads, the response then decreasing at higher doses. Many examples of this type of behaviour have been cited by UNSCEAR<sup>7</sup>, Mole<sup>8</sup> and ourselves<sup>9</sup>. The decrease in biological effect at high doses may arise for a variety of reasons ranging from loss of reproductive capacity<sup>8</sup> or 'cell death' to simple Poisson probabilities of events<sup>9</sup>. We have often used one form of response of particular importance, namely

$$\phi(p_c D) = p_c^2 D^2 \exp(-\lambda D) \quad (2)$$

as representative of a range of biological observations.

Only for uniform irradiation of an organ or tissue can we expect the observed macroscopic dose-response relationship to have the same form as the cellular dose-response function<sup>6</sup>.

## Point source $\beta$ and $\gamma$ emitters

We have integrated equation (1) using the cellular response function (2) for  $\beta$ -emitting single point sources of high and low energy<sup>6</sup>. The high energy source chosen was <sup>86</sup>Rb (91.2%, 1.78 MeV  $\beta$ ; 8.8%, 0.71 MeV  $\beta$ ), the small  $\gamma$  contribution being ignored. The low energy source was <sup>35</sup>S (100%, 0.167 MeV  $\beta$ ). For both sources, the dose-distance data tabulated by Cross<sup>9</sup> were used. The values of  $\lambda$  chosen ( $5 \times 10^{-4}$  and  $5 \times 10^{-3}$  rad<sup>-1</sup>) correspond to peaks in the dose-response function (2) at 4,000 and 400 rad respectively. The results of integration, performed numerically by computer, are shown in Fig. 1 as a function of the integrated source activity (Ci s).

When the same amount of energy is uniformly distributed throughout mass  $M$ , the corresponding expectation  $\epsilon_u$  for the same dose-response function (2) is

$$\epsilon_u = M n p_c^2 \bar{D}^2 \exp(-\lambda \bar{D}) \quad (3)$$

where  $\bar{D}$  is the mean organ dose.

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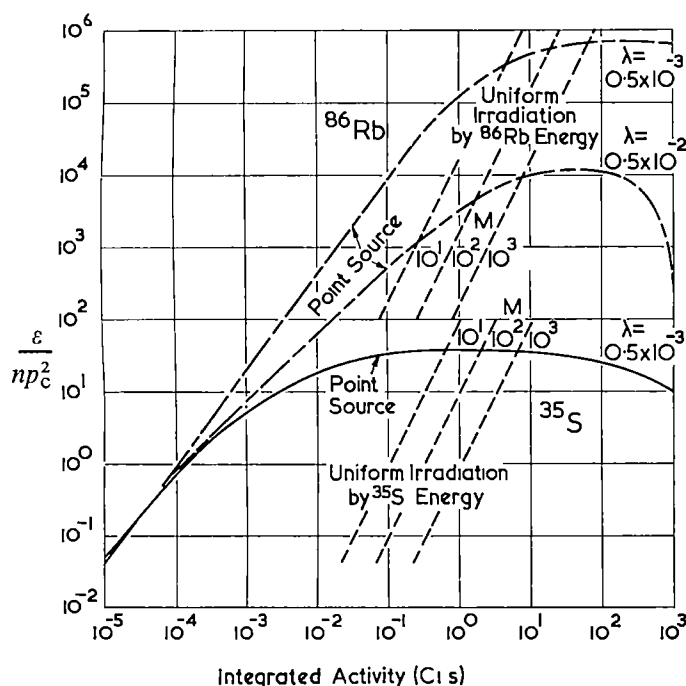


Fig. 1 Expectation of a malignancy from point sources of high ( $^{86}\text{Rb}$ ) and low ( $^{35}\text{S}$ ) energy  $\beta$  emitters, as a function of source activity compared with a uniform distribution of the same amount of energy through different masses  $M(\text{g})$ . The cellular response function was  $\phi(p_e D) = p_e^2 D^2 \exp(-\lambda D)$  for  $\lambda = 5 \times 10^{-4}$  and  $5 \times 10^{-3} \text{ rad}^{-1}$

The results of equation (3) are also plotted in Fig. 1, where the ordinate is not the expectation,  $\epsilon$ , but the related quantity  $\epsilon/np_c^2$ . We will return later to the evaluation of  $np_c^2$  required to transform the expectations from equations (1) and (3) to absolute values.

From Fig. 1 we see that for tissue masses of more than  $\sim 1 \text{ g}$  and for low source strengths, the point-source expectation exceeds that from uniform irradiation. At high source strengths, the opposite may be true. The mean organ dose at which the crossover occurs increases with the  $\beta$  energy of decay and decreases with increasing organ mass.

The effect of reducing the dose at which the peak in the response function occurs, that is, increasing  $\lambda$ , is to reduce greatly the single point-source expectation. During the rest of this paper, we have concentrated on the dose-response function peaking at 4,000 rad ( $\lambda = 5 \times 10^{-4} \text{ rad}^{-1}$ ) since it predicts the higher expectations.

We note that the ratio of point-source expectation to that for uniform distribution of the same amount of energy is greatest at very low source strengths, that is, for small values of absolute expectation. We see too that the point source expectation rises roughly linearly with source strength. This rather surprising result suggests that the total energy absorbed is still an important parameter.

We have next applied the theory to a mixed  $\beta$ - $\gamma$  emitter,  $^{60}\text{Co}$ , with  $\gamma$  rays of 1.17 and 1.33 MeV per disintegration and  $\beta$ -rays of 0.318 MeV. The resulting expectations are shown in Fig. 2 for a 1-kg sphere of tissue. We see that above  $10^{-1} \text{ Ci s}$  (0.12 rad), the  $\gamma$  contribution adds significantly to the single point-source expectation, so that uniform irradiation becomes more 'carcinogenic' only beyond  $10^2 \text{ Ci s}$  (120 rad to 1 kg). We note that both the absolute energy and the ratio of  $\gamma$  to  $\beta$  decay affects the mean dose at which uniform irradiation becomes more carcinogenic.

### Point source $\alpha$ emitters

The range of  $\alpha$  particles is much smaller than that for most  $\beta$ -radiation, and the resulting doses are, correspondingly,

confined to very localised volumes. The dose-distance function assumed for  $^{239}\text{Pu}$  was

$$D = 1.83 \times 10^{-6} \left( \frac{0.69}{r^2} + 7.22 \times 10^4 \right) \text{ rad per disintegration} \quad (4)$$

(J. R. Harvey, personal communication) where  $r$  is the distance from a point source, if less than the assumed mean range of the three  $^{239}\text{Pu}$   $\alpha$ -particles ( $r \leq 35.5 \mu\text{m}$ ). The results of integrating equation (1) for a single point source of varying strength, using the response function (2) peaking at 400 and 4,000 rad respectively, are shown in Fig. 3, together with the results for uniform distribution of the same amount of energy throughout 1 g of tissue.

Again we see that at very low source strengths there is an increased relative risk from a single point source of  $^{239}\text{Pu}$  compared with uniform irradiation. At higher source strengths the expectation falls more steeply than with a  $\beta$ -particle source because of the assumed form of the  $\alpha$  dose-distance function. Uniform irradiation becomes more carcinogenic beyond about  $3 \times 10^{-6} \text{ Ci s}$  for response functions peaking at 4,000 rad and beyond  $3 \times 10^{-7} \text{ Ci s}$  for those peaking at 400 rad. The effect of changes of value of  $\lambda$  in equation (2) are thus even more marked for  $\alpha$  sources than  $\beta$  emitters.

From Fig. 3 we see that, for a point source, at  $\geq 10^{-6} \text{ Ci s}$  'cell killing' predominates even for the smaller value of  $\lambda$ . One may question the use of our homogeneous dose function, when individual cells have highly ionising tracks passing through a small fraction of their volume. The detailed pattern of distribution of energy loss from an  $\alpha$  particle passing through a single cell may be important<sup>6</sup>, and the stochastic methods of microdosimetry developed by Rossi and Kellerer<sup>10</sup> may be more appropriate. We note, however, that our model suggests a peak expectation at about the same integrated source strength as predicted recently by Bair *et al.*<sup>11</sup> on a stochastic basis assuming one  $\alpha$ -particle traversal per cell.

We also note that our absolute estimates (below) of risk are of the same order as that deduced by Spiers and Vaughan<sup>12</sup> for the case of small numbers of cells subject to the risk of osteogenic sarcoma.

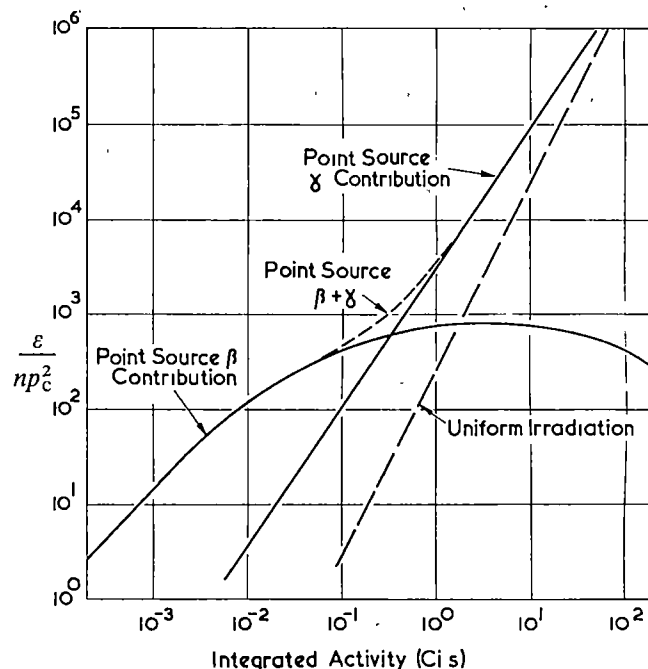


Fig. 2 Expectation in 1 kg of tissue from a point source of  $^{60}\text{Co}$  compared with a uniform distribution of the same amount of energy for a cellular response  $\phi(p_e D) = p_e^2 D^2 \exp(-0.5 \times 10^{-3} D)$

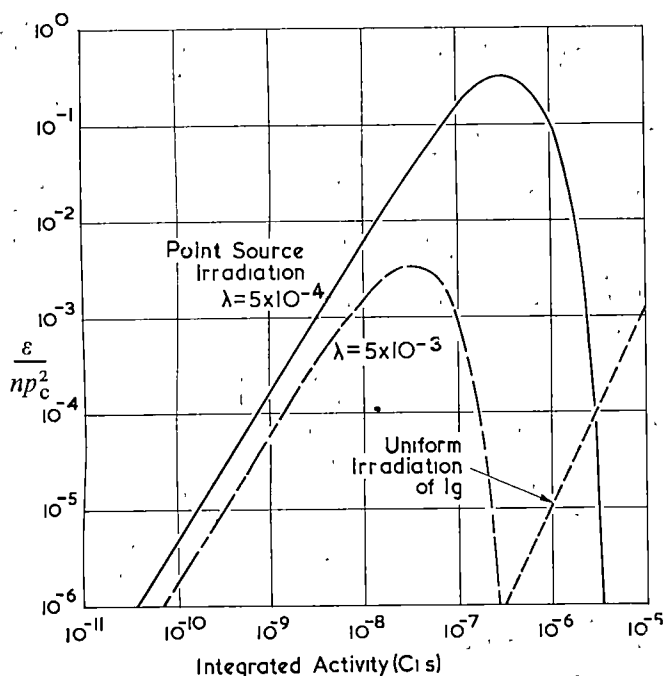
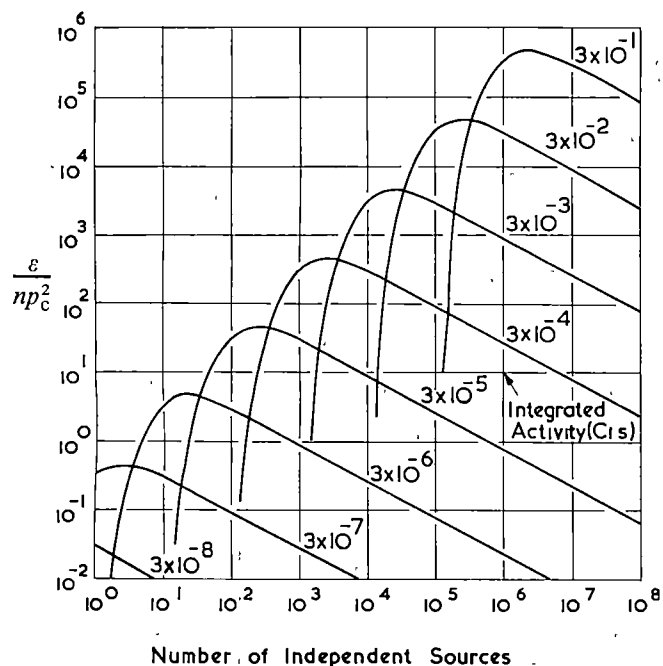


Fig. 3 Expectation from a point source of  $^{239}\text{Pu}$   $\alpha$  particles as a function of source activity compared with that from uniform irradiation of 1 g of tissue by the same amount of energy using a cellular dose-response function  $\phi(p_c D) = p_c^2 D^2 \exp(-\lambda D)$  for  $\lambda = 5 \times 10^{-4}$  and  $5 \times 10^{-3} \text{ rad}^{-1}$ .

### Fractionation of single point sources

Figure 4 shows the results of breaking up a single  $^{239}\text{Pu}$   $\alpha$ -emitting 'hot particle' into as many as  $10^8$  smaller particles, keeping the mean organ dose constant. We see that there is a maximum expectation when each particle activity approaches that corresponding to the peak of the single point source results of Fig. 3. Further fractionation decreases the expectation, since the differential coefficient of the single point source expectation, with respect to source strength  $< 10^{-7} \text{ Ci s}$ ,

Fig. 4 Expectation in a large mass of tissue for a given organ burden of  $^{239}\text{Pu}$  fractionated to varying numbers of independent sources. The cellular response function peaked at 4,000 rad.



is  $> 1$ . The maximum expectation increases roughly linearly with increasing mean organ dose.

Figure 5 shows the results of fractionation of the single  $\beta$ -emitting point source using the response function peaking at 4,000 rad. For the  $^{86}\text{Rb}$  point source, at low mean organ doses, the effect of increasing the number of sources while keeping the organ burden constant, is to reduce the risk. Above  $\sim 10 \text{ Ci s}$  (4 rad to 1 kg) there is a 'pessimism' number of particles. At higher doses significant dose overlapping occurs between sources. When the  $^{35}\text{S}$  single point source is fractionated, the expectation is increased for mean doses of more than  $3 \times 10^{-4} \text{ rad to 1 kg}$  ( $10^{-2} \text{ Ci s}$ ). We note the four orders of magnitude lower mean organ dose at which fractionation becomes important for the low, as compared with the high-energy  $\beta$  emitter.

### Moving point sources of $^{239}\text{Pu}$

As our model is assumed to be independent of dose rate, the problem of a moving point source is essentially that of a line source. We have previously presented a theoretical model for such  $\beta$  sources<sup>6</sup> and have now performed similar integrations for a  $^{239}\text{Pu}$   $\alpha$ -point source moving various distances through tissue. Neglecting end effects, the expectation for a source strength  $S_L$  per unit length (that is the integrated source strength/velocity of travel) is shown in Fig. 6. We see that a high source strength moving with high velocity can be equivalent in expectation to a low source strength at low velocity.

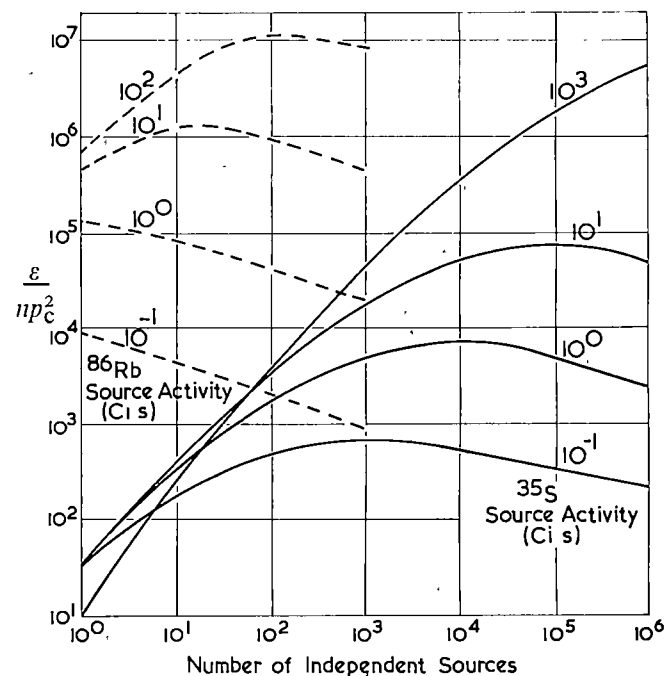


Fig. 5 Expectation in a large mass of tissue as a function of the number of discrete sources for a given organ burden of a high ( $^{86}\text{Rb}$ ) and low ( $^{35}\text{S}$ ) energy  $\beta$  emitter, assuming  $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$ .

These results again merely reflect the fact that the significant contributions to expectation originate largely from a narrow dose band around the maximum of the dose-response curve.

### Absolute values of expectation

The basic problem<sup>8</sup> lies in attempting to quantify  $p_c$ , the probability of transformation per cell per rad. We know that for a range of human body organs the risk of carcinogenesis is between  $\sim 0.1\%$  and  $1\%$  per 100 rad, for doses in the region of a few hundred rad (ref. 13). If a typical value of  $0.5\%$  per 100 rad be assumed, on the basis of a linear hypothesis the pro-

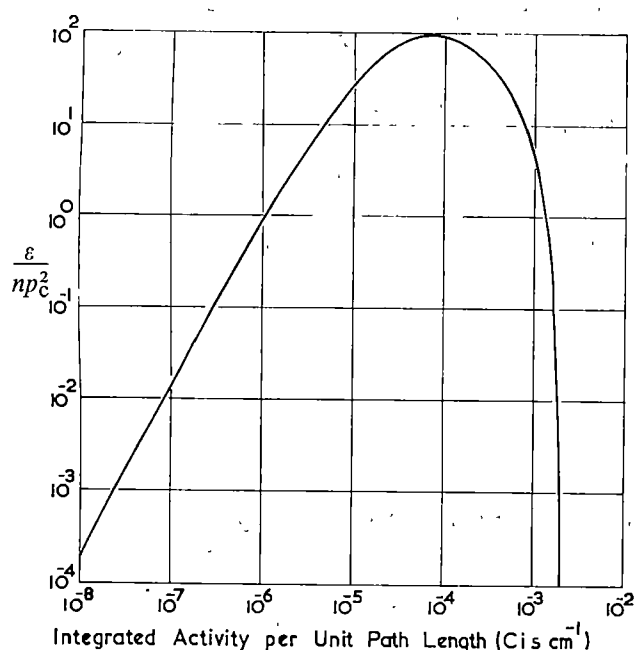


Fig. 6 Expectation from a moving point source of  $^{239}\text{Pu}$  using a cellular response function,  $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$ .

bability per tissue ( $p_t$  | linear) is

$$(p_t | \text{linear}) = 5 \times 10^{-5} \text{ rad}^{-1}$$

On a dose-squared hypothesis, using equation (2) and ignoring the exponential term at the normalisation dose of 100 rad

$$(p_t | \text{square}) = 7.1 \times 10^{-4} \text{ rad}^{-1}$$

Assuming an organ of mass  $M$  with  $n$  cells per unit mass it is easy to show that

$$(p_c | \text{linear}) = (p_t | \text{linear}) / Mn$$

$$(p_c | \text{square}) = (p_t | \text{square}) / (Mn)^{1/2}$$

For a 1-kg organ containing  $\sim 10^9$  cells per unit mass, we find

$$n(p_c | \text{square})^2 = 5 \times 10^{-10} \text{ g}^{-1} \text{ rad}^{-1}$$

On the linear hypothesis the value is

$$n(p_c | \text{linear}) = 5 \times 10^{-8} \text{ g}^{-1} \text{ rad}^{-1}$$

We note that normalisation at a higher dose would reduce the estimate of  $n(p_c | \text{square})^2$ .

Respirable 'hot particles' often have activities in the range 10 pCi–100 nCi. Let us assume a mean residence time in the lung of  $10^8$  s ( $> 3$  yr). The results then obtained for a single point source of  $^{86}\text{Rb}$  and  $^{35}\text{S}$  are shown in Table 1, where it is seen that the absolute expectation for a high energy  $\beta$  emitter ( $^{86}\text{Rb}$ ) from a point source using the square-law response function is essentially the same as that predicted by the linear hypothesis using mean organ dose. For a low energy  $\beta$  emitter (here  $^{35}\text{S}$ ), at higher source strengths, assumed linearity leads to higher values of the absolute predicted risks.

If the results were evaluated for 1 g of tissue, we should raise both  $n(p_c | \text{linear})$  and  $n(p_c | \text{square})^2$  by a factor of  $10^3$ . In Table 1 the mean organ doses and absolute expectations would also increase by a factor of  $10^3$ .

When fractionation is considered, at organ burdens  $< 10$  Ci s the  $^{86}\text{Rb}$  effects are reduced, so the use of mean organ doses and linearity overestimates risks. For  $^{35}\text{S}$ , however, fractionation increases expectation: at 10 Ci s an increase of  $10^3$  in expectation is seen on the square-law response. The effect of this on the  $^{35}\text{S}$  expectations in Table 1 is to bring the linear hypothesis and power-law estimates into close agreement.

The results for absolute expectations associated with stationary sources of  $^{239}\text{Pu}$   $\alpha$  particles are shown in Table 2. Since the single point-source results apply only at very low mean organ dose ( $< 10^{-6}$  Ci s  $= 3 \times 10^{-6}$  rad  $\text{kg}^{-1}$ ), expectations have been quoted for the distribution of activity leading to peak expectation following fractionation (Fig. 4). We see that

Table 2 Comparison of absolute expectation from  $^{239}\text{Pu}$   $\alpha$ -point sources fractionated to give peak expectation with the cellular response function  $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$ , and that predicted by linearity for a 1-kg organ and a given incidence of 0.5% at 100 rad

Integrated activity (Ci s)	$D$ to 1 kg (rad)	Absolute expectation	
		Peak value, nonlinear response	Linear response
$10^{-7}$	$3 \times 10^{-7}$	$7.5 \times 10^{-11}$	$1.5 \times 10^{-11}$
$10^{-3}$	$3 \times 10^{-3}$	$7.5 \times 10^{-7}$	$1.5 \times 10^{-7}$
$10^{-2}$	$3 \times 10^{-2}$	$7.5 \times 10^{-6}$	$1.5 \times 10^{-6}$
$10^{-1}$	$3 \times 10^{-1}$	$7.5 \times 10^{-5}$	$1.5 \times 10^{-5}$
$10^0$	$3 \times 10^0$	$7.5 \times 10^{-4}$	$1.5 \times 10^{-4}$
$10^1$	$3 \times 10^1$	$7.5 \times 10^{-3}$	$1.5 \times 10^{-3}$

for virtually all source strengths of interest in radiological protection, the peak of absolute expectation predicted by the power-law response is a factor of  $\sim 5$  above the value estimated by using mean organ doses and linearity. To obtain such expectations it has been necessary to choose circumstances when the  $^{239}\text{Pu}$   $\alpha$  energy is distributed in such a way that the largest number of cells are exposed to doses in the region of the peak of the dose-response curve.

The moving  $\alpha$ -particle source results from Fig. 6 have been transformed into absolute expectations in Table 3. Once again the use of linearity and mean organ doses predicts expectations

Table 1 Comparison of absolute expectation from a single point source of  $^{35}\text{S}$  or  $^{86}\text{Rb}$  in a 1-kg organ, as predicted using cellular responses  $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$  and  $p_c D$  (linearity) using different values of  $p_c$  derived for each response function assuming a given incidence of 0.5% per 100 rad

Nuclide	Integrated activity (Ci s)	$\epsilon / np_c^2$ (Fig. 1)	$D$ to 1 kg (rad)	Absolute expectation	
				Nonlinear response	Linear response
$^{86}\text{Rb}$ $E_\beta = 0.67$ MeV	$10^{-3}$	$2 \times 10^1$	$41.4 \times 10^{-4}$	$1.0 \times 10^{-8}$	$2 \times 10^{-8}$
	$10^{-2}$	$4 \times 10^2$	$4.14 \times 10^{-3}$	$2.0 \times 10^{-7}$	$2 \times 10^{-7}$
	$10^{-1}$	$8 \times 10^3$	$4.14 \times 10^{-2}$	$4.0 \times 10^{-6}$	$2 \times 10^{-6}$
	$10^0$	$1.2 \times 10^5$	$4.14 \times 10^{-1}$	$6.0 \times 10^{-5}$	$2 \times 10^{-5}$
	$10^1$	$4.4 \times 10^6$	$4.14 \times 10^0$	$2.2 \times 10^{-4}$	$2 \times 10^{-4}$
$^{35}\text{S}$ $E_\beta = 0.05$ MeV	$10^{-3}$	$5 \times 10^0$	$3 \times 10^{-5}$	$2.5 \times 10^{-9}$	$1.5 \times 10^{-9}$
	$10^{-2}$	$1.8 \times 10^1$	$3 \times 10^{-4}$	$9.0 \times 10^{-9}$	$1.5 \times 10^{-8}$
	$10^{-1}$	$3.3 \times 10^1$	$3 \times 10^{-3}$	$1.7 \times 10^{-8}$	$1.5 \times 10^{-7}$
	$10^0$	$3.8 \times 10^1$	$3 \times 10^{-2}$	$1.9 \times 10^{-8}$	$1.5 \times 10^{-6}$
	$10^1$	$3.8 \times 10^1$	$3 \times 10^{-1}$	$1.9 \times 10^{-8}$	$1.5 \times 10^{-5}$

**Table 3** Comparison of absolute expectation from a moving point source of  $^{239}\text{Pu}$   $\alpha$  activity using a cellular response  $\phi(p_e D) = p_e^2 D^2 \exp(-0.5 \times 10^{-3} D)$  and that predicted by linearity for a 1-kg organ and a given incidence of 0.5% per 100 rad

Source strength $S$ (Ci)	Velocity (cm s $^{-1}$ )	$S_L$ (Ci s cm $^{-1}$ )	Distance moved (cm)	Absolute expectation Nonlinear response (Fig. 6)	Linear response
$10^{-12}$	$10^{-1}$	$10^{-11}$	$10^{-2}$	$5 \times 10^{-21}$	$1.5 \times 10^{-17}$
			$10^1$	$5 \times 10^{-18}$	$1.5 \times 10^{-14}$
	$10^{-5}$	$10^{-7}$	$10^{-2}$	$7 \times 10^{-14}$	$1.5 \times 10^{-13}$
			$10^1$	$7 \times 10^{-11}$	$1.5 \times 10^{-10}$
$10^{-9}$	$10^{-1}$	$10^{-8}$	$10^{-2}$	$10^{-15}$	$1.5 \times 10^{-14}$
			$10^1$	$10^{-12}$	$1.5 \times 10^{-11}$
	$10^{-5}$	$10^{-4}$	$10^{-2}$	$5 \times 10^{-10}$	$1.5 \times 10^{-10}$
			$10^1$	$5 \times 10^{-7}$	$1.5 \times 10^{-7}$
	$10^{-6}$	$10^{-3}$	$10^{-2}$	$2.5 \times 10^{-11}$	$1.5 \times 10^{-9}$
			$10^{-1}$	$2.5 \times 10^{-8}$	$1.5 \times 10^{-6}$

in excess of those for the power-law response except when the source strength per unit length (or source strength/velocity) is at the peak of the curve in Fig. 6 (at  $\sim 10^{-4}$  Ci s cm $^{-1}$ ), when the power law predicts slightly higher expectations than the linear hypothesis. This is true for any combination of source strengths, velocities and distances, which again reflects the fact that peak expectations arise from the power-law response when the greatest number of cells are exposed to doses near the peak of the dose-response curve.

## Conclusions

We have estimated carcinogenic expectations by integrating nonlinear peaked cellular dose-response functions over spatially varying dose distributions and contrasted them with the expectations predicted by the linear hypothesis. If the dose-response function is peaked, then it is noticeable that the relative expectation is largely controlled by cells receiving doses in the vicinity of the peak. Thus, whether the relative maximum expectation arises from uniform irradiation, from a single point source, from a 'pessimum' number of 'hot particles', or during relative motion of the source, depends on the energy absorbed being distributed so as to subject the largest number of cells to doses near the peak of the response curve.

When we estimate absolute values of expectation, however, the use of the mean organ dose and the assumption of a linear non-threshold dose-response function seems to give a good guide to the upper limit of absolute expectation predicted

even for a peaked nonlinear function. Linearity can be very conservative for small numbers of  $\alpha$ - or low energy  $\beta$ -emitting sources, while at worst it seems unlikely to underestimate risks by more than a factor of  $\sim 5$  for those exceptional distributions of activity which lead to maximum expectations. The circumstances in which the 'hot particles' are relatively more dangerous are those in which the absolute risks are low.

An underestimation of the risk on assuming linearity would be most likely to occur if there was a high power-law response (say,  $D^4 \exp(-\lambda D)$ ) fitting the linear response at low doses ( $< 100$  rad) and the response peaking in the region of 10,000 rad rather than between 100 and 1,000 rad.

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# Aspects of the dosimetry of plutonium in bone

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*After animals have been exposed to plutonium it eventually becomes distributed unevenly throughout the bone tissue. There is therefore a need to study the dosimetry of plutonium, particularly  $^{239}\text{Pu}$ , as a function of its depth of burial in bone. This article discusses a simple approach to the problem, and the magnitude of some of the possible errors involved.*

PLUTONIUM is deposited primarily on the endosteal surfaces of cortical and trabecular bone<sup>1</sup> although a certain amount is also found on periosteal surfaces. After deposition the plutonium gradually moves into the volume of bone mainly by

becoming buried under newly deposited bone mineral<sup>1</sup>. Thus, at times long after a single intravenous injection, the distribution of plutonium in bone will typically consist of a thin layer of the element some distance below the endosteal surface together with a diffuse distribution between that layer and the endosteal surface.

Endosteal surfaces are defined here as the mineral bone surface of all marrow cavities and haversian canals; that is, the interface between mineralised bone and non-mineralised osteoid. Thus, if the skeleton contains  $1 \mu\text{Ci}$  of  $^{239}\text{Pu}$  and if  $A \text{ m}^2$  is the total area of endosteal surface, then  $1/A \mu\text{Ci}$  of the radionuclide will be associated with each  $\text{m}^2$  of the surface, if deposition on periosteal surfaces is neglected. If the plutonium is buried a distance,  $d \mu\text{m}$ , below the endosteal



Table 1 Calculated values  $10^2(k(q, d)/q)$ 

Depth, $d$ ( $\mu\text{m}$ )	5	10	15	20	25	30
0	3.10	2.53	2.20	1.94	1.74	1.57
1	$\pm 0.22$	$\pm 0.16$	$\pm 0.12$	$\pm 0.09$	$\pm 0.07$	$\pm 0.06$
2	2.78	2.36	2.04	1.80	1.60	1.42
3	$\pm 0.11$	$\pm 0.09$	$\pm 0.07$	$\pm 0.06$	$\pm 0.06$	$\pm 0.05$
4	2.24	1.98	1.75	1.56	1.38	1.22
5	$\pm 0.11$	$\pm 0.08$	$\pm 0.06$	$\pm 0.05$	$\pm 0.04$	$\pm 0.03$
6	2.14	1.89	1.65	1.45	1.28	1.14
7	$\pm 0.11$	$\pm 0.09$	$\pm 0.07$	$\pm 0.05$	$\pm 0.05$	$\pm 0.04$
10	1.97	1.82	1.61	1.44	1.27	1.11
15	$\pm 0.10$	$\pm 0.09$	$\pm 0.07$	$\pm 0.05$	$\pm 0.05$	$\pm 0.04$
20	1.84	1.68	1.50	1.31	1.12	0.96
25	$\pm 0.05$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$
30	1.64	1.43	1.30	1.14	0.98	0.82
35	$\pm 0.06$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.04$	$\pm 0.03$
40	1.70	1.46	1.25	1.08	0.91	0.76
45	$\pm 0.10$	$\pm 0.08$	$\pm 0.06$	$\pm 0.05$	$\pm 0.04$	$\pm 0.04$
50	1.22	1.07	0.93	0.79	0.64	0.53
55	$\pm 0.05$	$\pm 0.06$	$\pm 0.05$	$\pm 0.04$	$\pm 0.03$	$\pm 0.03$
60	0.74	0.58	0.44	0.33	0.26	0.22
65	$\pm 0.04$	$\pm 0.04$	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$
70	0.31	0.17	0.12	0.09	0.07	0.06
75	$\pm 0.04$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.01$	$\pm 0.01$

Errors are 1 s.d. statistical errors arising from the limited number of Monte Carlo iterations used.

surface then the dose rate,  $D_s(q, d)$  to an endosteal tissue layer of thickness  $q$   $\mu\text{m}$  will be given by

$$D_s(q, d) = I \times E_\alpha \times 51.2 \times k(q, d) / Aq \rho \text{ rad d}^{-1} \quad (1)$$

where  $E_\alpha$  is the energy of the  $^{239}\text{Pu}$   $\alpha$  emission in MeV,  $\rho$  is the density of soft tissue, which may be taken as  $1 \text{ g cm}^{-3}$ , and  $k(q, d)$  is the average fraction of that energy absorbed in an endosteal tissue layer of  $q$   $\mu\text{m}$  thickness if the decay occurs  $d$   $\mu\text{m}$  below the endosteal surface.

It is usual to define  $D_{\text{BONE}}$  as the average dose rate to an infinite extent of mineral bone contaminated at a level of  $I/M \text{ } \mu\text{Ci g}^{-1}$ ,  $M$  being the mass of mineral bone in grams. Thus

$$D_{\text{BONE}} = 51.2 \times I \times E_\alpha / M \text{ rad d}^{-1} \quad (2)$$

From equations (1) and (2) it can be seen that

$$D_s(q, d) / D_{\text{BONE}} = Mk(q, d) / Aq \rho \quad (3)$$

For man,  $M$  may be taken as 5,000 g, since the evidence suggests that bone mineral comprises about 7% of the total adult body weight<sup>2</sup>. The value of  $A$  has been variously estimated at 14.0 (ref. 2), 12.5 (J. M. Marshall *et al.*, unpublished) and 16 m<sup>2</sup> (F. W. Spiers, personal communication). I shall take  $A$  at 12.5 m<sup>2</sup> in accordance with the results presented by Marshall (unpublished), but it should be emphasised that this is an estimate, and that the true mean for a particular occupationally exposed population could easily differ from this by as much as 25%.

Inserting these values into equation (3) gives

$$D_s(q, d) / D_{\text{BONE}} = 400k(q, d) / q \quad (4)$$

The value of  $k(q, d)$  has been calculated using Monte Carlo techniques under two simplifying assumptions: first, that bone

surfaces are essentially flat over distances comparable with the range of an  $\alpha$  particle in tissue; second, that the surfaces are sufficiently far apart for cross-fire to be neglected. Decays were originated at a distance  $d$   $\mu\text{m}$  below the endosteal surface, and emission directions were chosen at random to yield an isotropic decay distribution. Using a modified form of the Bragg equation the rate of energy loss along each of the randomly chosen tracks was evaluated and the fraction of energy deposited in the first  $q$   $\mu\text{m}$  above the endosteal surface was thus determined.

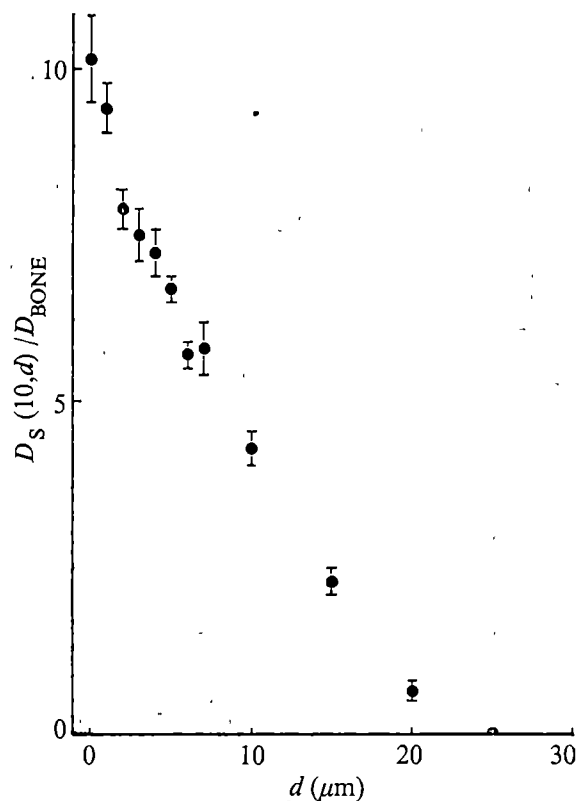


Fig. 1 Relationship between the dose rate to the endosteal layer and the depth of burial of plutonium in bone mineral.

A considerable amount of approximation was involved in determining the rate of energy loss along each track. For soft tissue the form of the Bragg equation was taken from Harley and Pasternak<sup>3</sup> except that an  $\alpha$ -particle shielding correction was used at energies less than 2 MeV, in order that the calculated values of stopping power in this energy range were in agreement with those tabulated by Walsh<sup>4</sup>. That gave a  $^{239}\text{Pu}$   $\alpha$ -particle range in soft tissue of 37  $\mu\text{m}$ . The same Bragg equation was used for mineralised bone, except that, at all  $\alpha$ -particle energies, the stopping power was multiplied by a constant,  $C$ , given by

$$C = (\rho_{\text{BONE}} / \rho_{\text{SOFT TISSUE}}) (SP_{\text{BONE}} / SP_{\text{SOFT TISSUE}}) \quad (5)$$

The value for  $\rho_{\text{BONE}}$ , the density of mineral bone, was taken to be  $1.95 \text{ g cm}^{-3}$  (ref. 2) and that of  $\rho_{\text{SOFT TISSUE}}$  was taken as unity. The ratio of the mass stopping power of soft tissue,  $SP_{\text{SOFT TISSUE}}$  to the mass stopping power of bone,  $SP_{\text{BONE}}$ , is not well known. It has been estimated at 1.225

Table 2 Values of  $D_A(d) / D_{\text{BONE}}^*$ 

$d(\mu\text{m})$	0	1	2	3	4	5	6	7	10	15	20	25
0.83	0.73	0.62	0.57	0.56	0.48	0.41	0.38	0.27	0.11	0.029	0.00	—
—	$\pm 0.03$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.02$	$\pm 0.01$	$\pm 0.003$	—

\*Errors are statistical errors from the number of Monte-Carlo iterations used.

(ref. 5) and 1.41 (J. M. Marshall, unpublished), depending on the degree of calcification assumed. The ratio is taken here as 1.3, but that value could easily be in error by as much as 10%. It is for this reason that the same Bragg curve has been used for both mineral bone and tissue, since any difference depending upon the different elemental composition of mineral bone and soft tissue would be small compared with the estimated systematic error of 10%.

Using the Monte Carlo calculations  $k(q, d)/q$  was calculated for various values of  $q$  and  $d$  (Table 1).

If  $q$  is taken as 10  $\mu\text{m}$ , a value suggested<sup>6</sup> for evaluating the dose to cells on endosteal surfaces, it can be seen from Fig. 1 that the relationship between the dose rate to the endosteal layer and the depth of burial of the plutonium is slightly curvilinear. Over a range of depths of burial from 2  $\mu\text{m}$  to 20  $\mu\text{m}$  this curvilinear relationship may, however, be closely approximated by a straight line. Thus, for plutonium distributions in which the bulk of the plutonium lies within this range, the average depth of burial may be used to calculate the average endosteal dose.

The methods described here have also been used to calculate the average dose rate to active red bone marrow from plutonium buried at different depths in mineral bone. In the adult human, active red bone marrow is primarily associated with the trabecular marrow spaces, and is estimated to have a mass of 1,500 g (ref. 2). If  $I_T \mu\text{Ci}$  of  $^{239}\text{Pu}$  are associated with trabecular bone and  $I_C \mu\text{Ci}$  are associated with cortical bone, then the average dose rate to active red bone marrow,  $D_A(d)$ , from  $^{239}\text{Pu}$  buried a distance  $d \mu\text{m}$  below the endosteal surface is given by

$$D_A(d) = 51.2 I_T E_a k(q_A, d) / 1,500 \text{ rad d}^{-1} \quad (6)$$

where  $q_A$  is a value of  $q$  greater than the range of  $^{239}\text{Pu}$   $\alpha$  particles in soft tissue. Since

$$D_{\text{BONE}} = 51.2(I_C + I_T)E_a/5,000 \text{ rad d}^{-1} \quad (7)$$

It follows that

$$D_A(d)/D_{\text{BONE}} = (10/3)(I_T/(I_C + I_T))k(q_A, d) \quad (8)$$

If  $^{239}\text{Pu}$  is uniformly distributed over all endosteal surfaces, then  $I_C$  will be approximately equal to  $I_T$ , since the endosteal areas of cortical and trabecular bone are approximately equal<sup>3</sup>. Under this assumption

$$D_A(d)/D_{\text{BONE}} = (5/3)k(q_A, d) \quad (9)$$

Values of  $D_A(d)/D_{\text{BONE}}$  calculated according to this formula are given in Table 2 for various depths of burial. It should be noted that if the plutonium forms a thin layer exactly on endosteal surfaces then, by simple geometric arguments,  $k(q_A, 0) = 0.5$  and  $D_A(0)/D_{\text{BONE}} = 0.83$ .

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# Neutralised colliding beam torus

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*We describe a new type of deuterium-tritium fusion reactor, consisting of two dense oppositely-directed D and T plasma components confined in a torus. These components can be formed by neutral-beam injection at moderate energies. The reactor could have significant power gain with relatively lenient plasma confinement requirements.*

EXPERIMENTS over the past few years have demonstrated that a deuterium plasma at temperatures of  $\sim 1$  keV can be stably produced and confined in a torus. These experiments, which are directed toward producing a self-sustained controlled thermonuclear reactor, involve temperatures within one order of magnitude of practical reactor values. It is now reasonable to take seriously other proposals for toroidal devices, not necessarily self-sustained, which could conceivably be constructed sooner and which could produce net power.

## Two-component torus

One such possibility, which has been generally accepted as feasible, is the two-component torus (TCT)<sup>1-4</sup>. One component of the TCT is a cold tritium plasma (T), which serves as the target for the other component, an energetic confined beam of deuterons (D). The optimal deuteron injection energy is 150-200

keV (somewhat above the peak of the nuclear cross section) to increase the total probability of fusion, integrated over the deceleration of the deuterons by Coulomb interactions with the tritons and the neutralising plasma electrons. If the electron temperature is  $\gtrsim 5$  keV, it can easily be shown that the fusion power output exceeds the beam power injected into the plasma<sup>1-4</sup> (although the electrical power output is still less than the beam power). The electron temperature is maintained against transport and radiation losses by energy transfer from the energetic deuterons. The critical confinement of the electron energy and of the fast deuterons in such a device can be an order of magnitude poorer than confinement times required for a conventional self-sustaining reactor<sup>1-4</sup>, and plans are well advanced to build a prototype TCT.

## Colliding beam torus

Here we discuss another type of beam-driven toroidal reactor. We envisage a toroidal plasma in which the D and T ion components both have substantial energies,  $\sim 40$  keV, but move in opposite directions. The ion distributions are maintained by oppositely directed neutral beams of D and T which are injected into the torus and trapped by ion impact, charge exchange, and electron ionisation in the already-existing plasma of energetic D and T components (in steady state). Because of the opposite velocities of the D and T, the relative collision energy is nearly 4 times the energy of each beam, so that it is above the peak of

the D-T cross section ( $\sim 120$  keV). This is analogous to using colliding beams in high energy particle accelerators, but with plasmas the densities can be made much larger by continual trapping of the beams in the torus, and collisions occur throughout the entire volume of the torus. We estimate that densities  $\sim 5 \times 10^{13} \text{ cm}^{-3}$  may be achieved for realistic toroidal parameters with a few neutral-beam sources of already available types.

We denote the concept of two energetic colliding components as the 'colliding beam torus' or CBT. Its primary advantage over the TCT is the lower beam voltage required. Highly efficient generation of 40-keV neutral beams is already possible, while efficient generation of 150-keV beams is still in an embryonic state. Another advantage of the CBT is that the number of electrons per energetic ion is lower, so that the same electron temperature may be achieved with even poorer confinement of the electron energy than required by the TCT. The lower temperature and injection energy result in smaller plasma size.

### Model of the CBT

Let us consider how the CBT plasma may be set up. To trap the injected neutral beams, one must initially have a conventionally formed target plasma in the torus. Injection of the neutral D and T beams into the plasma must gradually replace the cooler plasma with the two energetic components, and finally, as the energetic components themselves slow down on the neutralising background of electrons, the lower energy parts of the beams must be removed much faster than the more energetic parts of the beams. We require that there be very few impurity ions in the torus, since impurities enhance angular scattering of the energetic ions, thereby destroying their directedness. More precisely, we hypothesise that first, the initial target plasma leaves and does not return, second, the plasma remains very pure, and third, the low energy ions leave much faster than the high energy ions. In our calculations on the CBT, we neglect entirely the loss of ions with energies  $W > 2T_e$ , and assume that the loss rate for  $W < 2T_e$  is constant, with a half life  $\tau$ . Under these hypotheses, we show by detailed calculations that the CBT situation can actually be maintained in steady state by beam injection at moderate energies. Further, we show that the resulting fusion power is quite favourable, relative to the injected-beam power, and that the energy confinement requirements are quite moderate. We indicate that the colliding plasma components can be formed by neutral injection without undue losses. Finally, calculations show that the CBT is stable to velocity-space instabilities.

There is some experimental evidence that our hypotheses can be realised. Experiments in the ATC tokamak show that, by gettering the walls, plasma leaving the torus can be captured<sup>5</sup>. The remaining plasma is very pure, and its density will decay nearly to zero, as required for the initial background plasma of the CBT. Similar results have been obtained in Alcator by special discharge cleaning<sup>6</sup>. We hope that similar effects could be produced in larger devices by efficient divertors. Further, beam injection into several tokamaks has been carried out, and the loss time of beam ions is found to be much larger than that of the thermal plasma<sup>7-9</sup>. Presumably, the losses are produced by micro-instabilities, and small-scale irregularities affect slow ions more than fast ions.

### Calculations

Consider an homogeneous plasma of D and T ions and an equal density of electrons in a uniform magnetic field,  $\mathbf{B}$ . Let the electrons have a Maxwellian distribution with temperature  $T_e$  determined by energy balance. Let there be equal monochromatic sources  $S/2$  of D and T at  $+v_{20}\mathbf{B}/|\mathbf{B}|$  and  $-v_{30}\mathbf{B}/|\mathbf{B}|$  respectively, and let both ion species be uniformly lost for energies  $< 2T_e$  at a rate  $\tau^{-1}$ . Then, for any  $T_e$ , a uniform steady state will be reached under the influence of D-T, D-e, and T-e

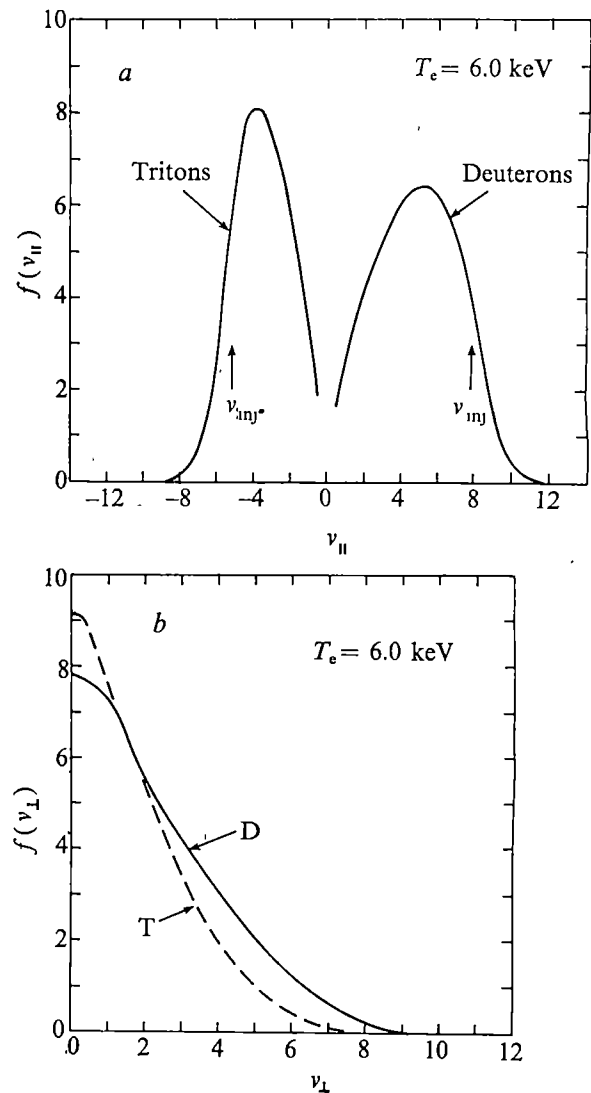


Fig. 1 Steady-state ion velocity distribution functions (a) parallel and (b) in a direction,  $\mathbf{x}$ , perpendicular to  $\mathbf{B}$ . The velocity scale is in  $(\text{keV})^{1/2}$  for the deuterons only. Injection velocities are indicated by  $v_{inj}$ ,  $n_T/n_D = 0.92$ .

Coulomb collisions. To determine the resulting three-dimensional distribution functions in velocity space for D and T,  $f_2(v_2)$ ,  $f_3(v_3)$ , we solve two simultaneous Fokker-Planck equations numerically, using one of the standard codes suitably modified to include two species of ions<sup>10</sup>. The finite size of the torus is simulated by an energy loss rate  $\tau_E^{-1}$  produced by thermal conductivity, radiation, and so on. The electron temperature  $T_e$  is determined by a power balance between the rate of energy gain by D-e and T-e collisions and the loss rate  $\tau_E^{-1}$ . In steady-state conditions

$$\frac{dT_e}{dt} = C_2(W_2 - 3/2T_e) + C_3(W_3 - 3/2T_e) - T_e/\tau_E = 0 \quad (1)$$

where  $W_2$  and  $W_3$  are the mean D and T energies, and  $C_2$  and  $C_3$  are constants. We use equation (1) to determine the  $\tau_E$  required to achieve a given  $T_e$ . Since  $\tau$ , the time constant for cool-ion loss, is somewhat arbitrary, we can make  $\tau_E$  and  $\tau$  equal. (The results are, however, relatively insensitive to  $\tau/\tau_E$  in the range 0.5–2.0.)

The resulting distribution functions for  $T_e = 6.0$  keV are shown in Figs 1 and 2 for injection energies  $W_{20} = 60$  keV for D and  $W_{30} = 40$  keV for T. Figure 1a shows the distribution of the parallel component of D and T and Fig. 1b the distribution

in the cartesian component  $v_x$ , where  $x$  is perpendicular to  $\mathbf{B}$ . Figure 2 shows the contours of the axisymmetric three-dimensional distribution functions in coordinates  $v_{||}$  and  $v_{\perp}$ , and we see that two oppositely moving axisymmetric ion components are formed. Further, the relative velocities in each beam are small, so that self-collisions dominate and the two components have, approximately, shifted Maxwellian distributions. Although the distribution in pitch angle  $\mu \simeq \tan^{-1}(v_{\perp}/v_{||})$  is not negligible, angular spread does not disturb the oppositely directed character of the two beams.

### Power generated

To find the relative gain in fusion power to beam power, we compute the ratio

$$Q = \frac{E_r}{0.5S(W_{20} + W_{30})} \int f_2(\mathbf{v}_2) f_3(\mathbf{v}_3) \sigma_{DT} \times \quad (2)$$

$$\times \sigma_{DT}(|\mathbf{v}_2 - \mathbf{v}_3|) |\mathbf{v}_2 - \mathbf{v}_3| d\mathbf{v}_2 d\mathbf{v}_3$$

where  $\sigma_{DT}$  is the D-T nuclear fusion cross section, and  $E_r \equiv 17.6$  MeV is the energy released per fusion. The results are given as a function of  $T_e$  in Fig. 3a. In Figure 3b we give the

Fig. 2 Steady-state contours of the ion speed distribution function in DT. The deuterons (a) are injected at 60 keV,  $\theta = 4^\circ$ , and the tritons (b) at 40 keV,  $\theta = 176^\circ$ .  $f(\mathbf{v})$  is normalised to the value at the source. Ions decelerated to energies  $< 2T_e$  are assumed to have a lifetime  $\tau \simeq \tau_E$ , as given in Fig. 3b. Velocity units are relative.

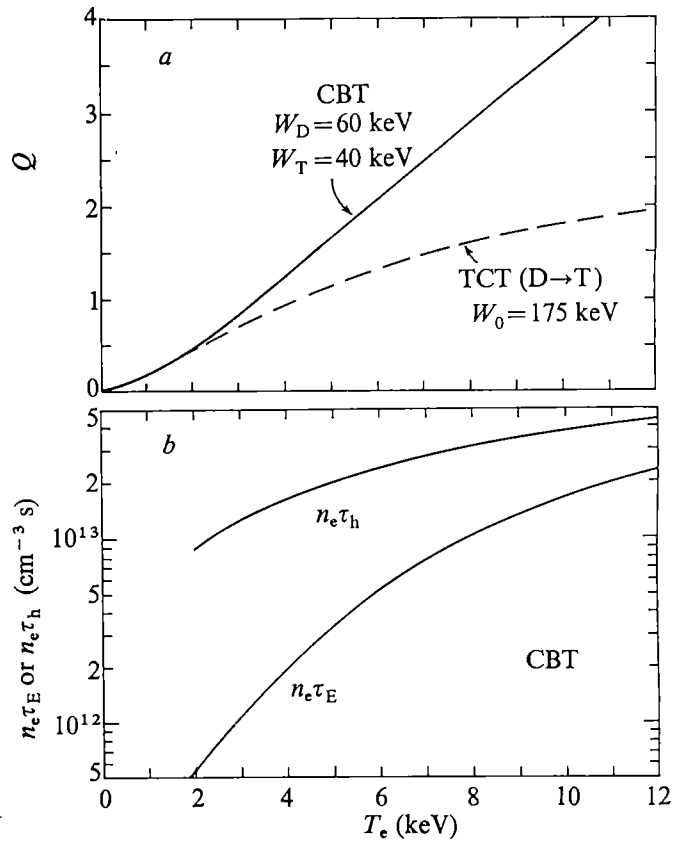
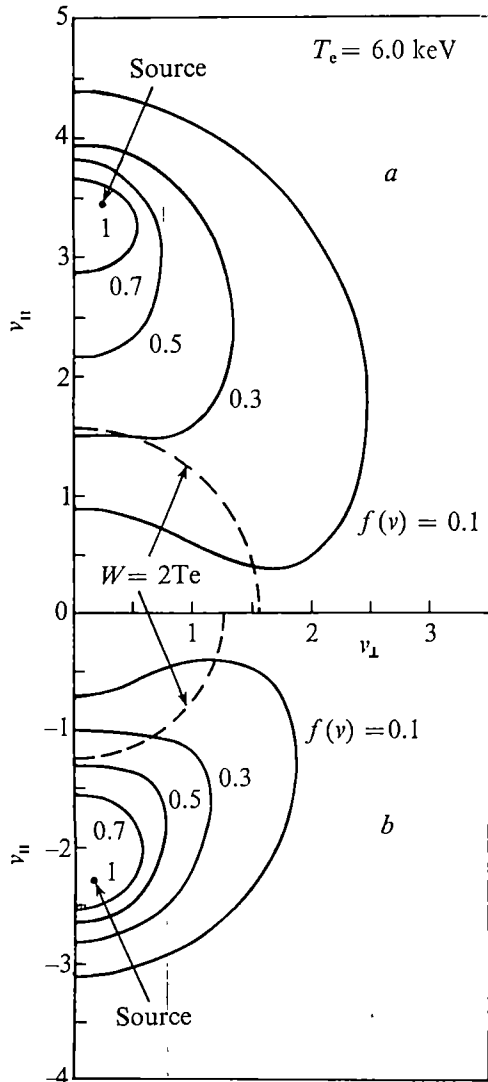


Fig. 3 a, Fusion energy gain  $Q$  of the CBT for steady-state operation in DT (17.6 MeV per reaction) b, The confinement parameters ( $n_e \tau_E$  and  $n_e \tau_h$ );  $\tau_h$  is the total lifetime of injected ions,  $\tau_E$  is the electron energy confinement time, as well as the lifetime of ions after deceleration below  $2T_e$ .

confinement parameter  $n_e \tau_E = n_e \tau$  required for these  $T_e$ . It is seen that  $Q$  is larger than unity for electron temperatures in excess of 3.5 keV, and that the required  $n_e \tau_E$  for 'breakeven' is  $1.5 \times 10^{12} \text{ s cm}^{-3}$ . The  $Q$  values for the TCT are given for comparison. Also shown is  $\tau_h = (n_2 + n_3)/S$ , the required lifetime of the energetic ions; this value is appreciably larger than  $\tau_E$ .

In order that the reactor have net electrical power output,  $Q$  must satisfy the relationship  $Q > (\eta_b \eta_i)^{-1} - 1$ , where  $\eta_b$  is the efficiency of neutral-beam production, and  $\eta_i$  is the energy conversion efficiency in the blanket cooling system. Taking  $\eta_i = 0.35$ , and  $\eta_b = 0.7$  (which is quite feasible for low-energy beams<sup>21</sup>), we require  $Q > 3.0$ , so that  $T_e \geq 8.5$  keV, and  $n_e \tau_E \geq 1.3 \times 10^{13} \text{ cm}^{-3} \text{ s}$  (Fig. 3). A practical power producer may have to operate at somewhat larger values of  $n_e \tau_E$ . These conditions can, however, be greatly relaxed if a fissionable blanket is used, and even at  $Q \simeq 1$ , the device could be useful as a fusion test reactor.

In this analysis, we have neglected the effect of 3.5-MeV alpha particles resulting from fusion. Confined alphas will give up their energy almost entirely to the electrons, thereby allowing the same  $T_e$  to be obtained with a smaller  $n_e \tau_E$ . The required  $n_e \tau_E$  is reduced from the value in Fig. 3 by the factor  $(1 + 0.2\alpha Q)^{-1}$ , where  $\alpha$  is the fraction of alphas that slow down in the plasma.

### Plasma formation and stability

We now consider the formation of the energetic-ion distributions. Neutral beams of D and T are injected into the torus, and ionised. The relative velocity of the incoming D beam is small compared with the  $D^+$  velocity, so that charge exchange on  $D^+$  and ion impact ionisation on  $T^+$  are probably about equal. Charge exchange leads to an energetic secondary neutral of roughly half the energy of the injected neutral. Its loss would



reduce the efficiency of beam trapping by 25%, but estimates show there is a good chance that it too will be trapped by ion impact. Thus, the efficiency of trapping is probably reduced by not more than 10%.

The stability of the CBT plasma has been examined against homogeneous electromagnetic and electrostatic waves. We approximate the equilibrium by two shifted Maxwellian distribution of identical ions. We find stability against all electrostatic instabilities not in the neighbourhood of the ion cyclotron frequency, if the random temperature of each beam exceeds  $T_e/2$ , a criterion well satisfied by the CBT distribution functions. Thus the CBT is stable against the two-stream instability. The analysis of electrostatic instabilities near the ion cyclotron frequency is more complex, but detailed calculations indicate that the CBT is stable against both electrostatic and electromagnetic waves in this frequency range (F. W. Perkins, unpublished). Finally, a simple analysis approximating the beams by delta functions indicates stability against all electromagnetic modes not near the cyclotron frequency if the plasma energy density is smaller than the energy density of the confining magnetic field—a condition always satisfied. It appears, therefore,

that the steady-state CBT system is stable to all velocity-space modes.

We thank Y. C. Sun for carrying out the numerical Fokker-Planck calculation. We thank Dr H. P. Furth for many stimulating discussions, and also acknowledge helpful conversations with Drs H. L. Berk, J. Ostriker, and F. W. Perkins. This work was supported by US Energy Research, and Development Administration.

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# letters to nature

## Anti-correlated hard and soft X-ray intensity variations of the black-hole candidates Cyg X-1 and A0620–00

EXTENDED spectral measurements are now available at more than one time epoch for the powerful Monoceros transient, A0620–00, discovered by Elvis *et al.*<sup>1</sup>, and the binary source Cyg X-1. We here point out a basic similarity in the unusual spectral time variations of these sources. Both are black-hole candidates, Cyg X-1 by virtue of its orbital dynamics and A0620–00 as suggested by arguments based on the Eddington limit<sup>1</sup> and distance estimates of the optical counterpart. Thus the X-ray emission mechanism could be common.

The type of data obtained by the Ariel V satellite are summarised in Table 1. All the detectors use some form of spin modulation to eliminate background. In particular, measurements with the ST detector are made at two directions offset from the source position given by the RMC. The observed phase of the modulation is then used to confirm that the signal is being correctly detected.

Observations at a few keV indicate that Cyg X-1 switches between two states: a normal state at a low intensity, the

'low state', and an enhanced or 'high state'. Around 1975 May 9 a 'high' to 'low' transition was observed (see ref. 2). Figure 1 shows ST data for the 'high' state taken during May 4–10 and for the 'low' state during May 11–15. The error bars correspond to 1 s.d., determined by counting statistics, which dominates calibration and other systematic errors. Also shown are the CPC data given in ref. 2 for the 'high' and 'low' state. Note that while below ~ 7 keV the 'high' state indeed corresponds to a higher intensity, the change in intensity at higher energies, extending out to at least 150 keV, is in the opposite sense. During the 'low' state a power law

$$\frac{dN}{dE} = 3.3 E^{-1.81} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

(dashed line) represents most of the data, while during the 'high' state, a more complicated spectral form is required.

Qualitatively similar time variations are noticed for A0620–00, both within a restricted low-energy range during the initial turn-on (Ricketts *et al.*<sup>3</sup>), and now over a much wider energy range, as indicated by the ST observations during the decay phase. Figure 2 shows spectra at two epochs.

Table 1 Ariel V data

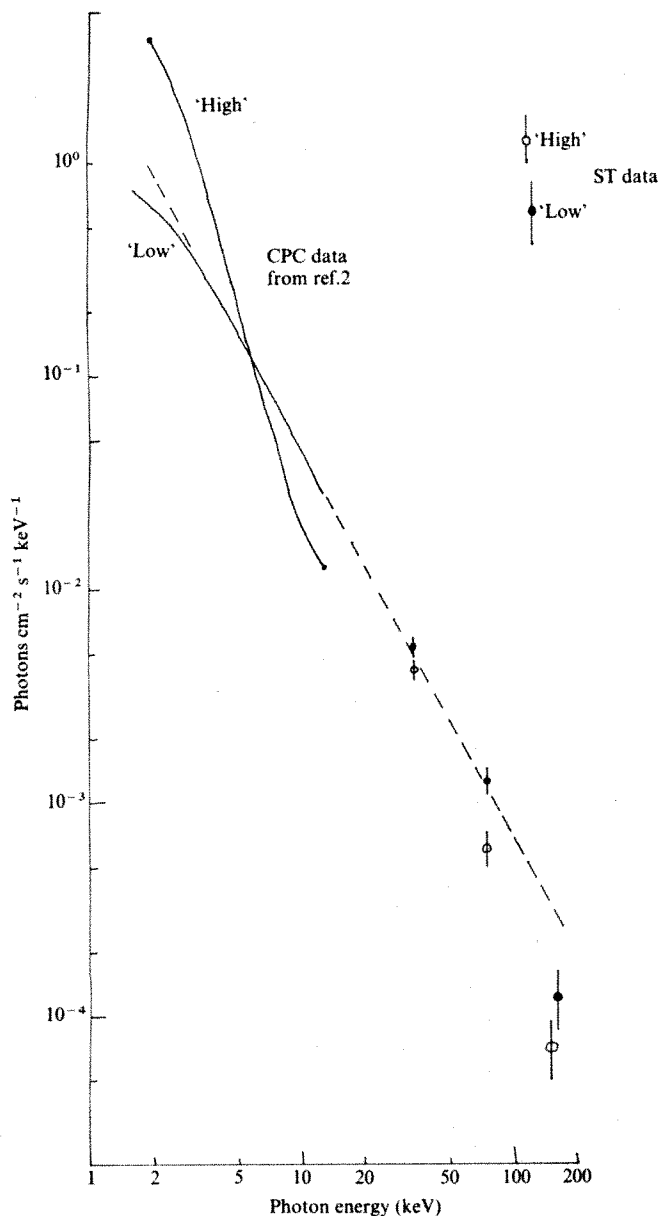
Detector and abbreviation	Area (cm <sup>2</sup> )	Detector axis tilt to satellite spin axis	Energy range (keV)	Institute and reference
CsI scintillator telescope (ST)	8	3°	26–1200	Imperial College Space Physics Group
Rotation modulation collimator (RMC)	102	0°	1.9–12	MSSL/Birmingham Dr G. Carpenter X-ray Astronomy Group, Birmingham University (personal communication)
Collimated proportional counter (CPC)	100	1.75°	1.5–20	Mullard Space Science Lab. Sanford <i>et al.</i> <sup>2</sup>
Sky survey experiment (SSE)	290	≈ 90°	2–18	Leicester University X-Ray Astronomy Group Ricketts <i>et al.</i> <sup>3</sup>

The first is 1975 mid-August when A0620-00 was near maximum intensity. The SSE data for August 22 and ST data for August 20-23 shown in Fig. 2 can be fitted with a single power law spectrum (solid line)

$$\frac{dN}{dE} = 3.5 \times 10^3 \times E^{-4.7} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

up to  $\sim 60$  keV, with an indication of hardening  $> 30$  keV to an exponent of  $\sim -2$ . Data for the second epoch in Fig. 2 are from the period October 16-19 for the ST detector, and October 19 for the RMC detector. Error bars for the ST data are again 1 s.d. from counting statistics, and systematic errors are expected to be small compared with this. We now note that whereas the mid-October soft X-ray intensity has decreased by a factor 5 or more, the 20-200 keV intensity seems to have increased by at least a factor 2. Moreover, a spectral break with two power laws is now definitely required to satisfy the totality of the October data. We suggest that the similar

**Fig. 1** Cyg X-1 X-ray spectra before and after the 1975 May 9 'high' to 'low' state transition measured by Ariel V detectors. The solid curves represent the data of Sanford *et al.*<sup>2</sup> while the dashed curve represents a power law fit.

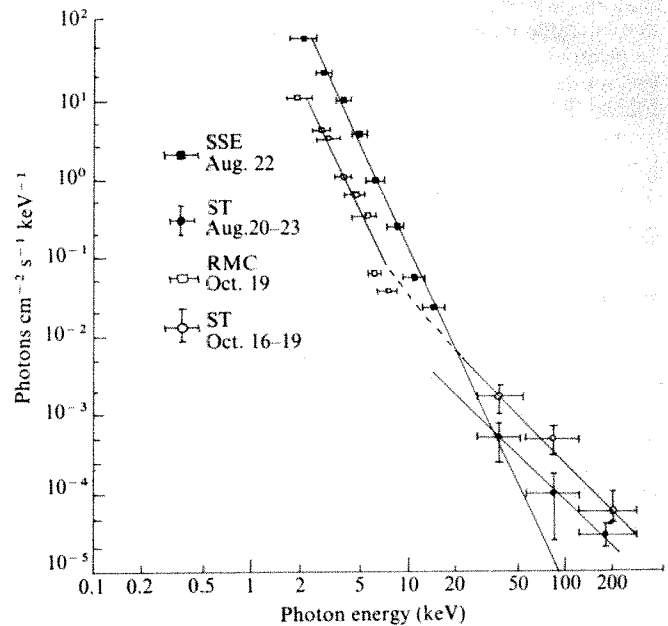


spectral transitions illustrated in Figs 1 and 2 indicate comparable production mechanisms for Cyg X-1 and A0620-00.

In seeking to understand the observed time variations we suggest that the inverse Compton source model of Eardley *et al.*<sup>4</sup> for Cyg X-1, and by implication A0620-00, could provide a plausible mechanism. Carpenter *et al.*<sup>5</sup> suppose acceleration in an optically thin region of a source containing a hot electron gas operates like the Fermi mechanism<sup>6</sup>, with a fractional photon energy change per collision

$$\frac{dE}{E} \approx \frac{v^2}{c^2}$$

for electron velocity  $v$ . If the probability of escape of photons from the acceleration region, after  $N$  collisions, is proportional



**Fig. 2** A0620-00 X-ray spectra in mid-August 1975 and mid-October 1975, observed by Ariel V detectors. Various power law fits are indicated by the solid lines.

to  $\exp(-N/k)$ , where  $k$  is a constant, a power law photon spectrum will result. Changes in the low energy photon supply and in the photon diffusion parameters which could vary in different acceleration regions may account for the observed slope changes and breaks in the spectra of Figs 1 and 2.

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## X-ray transient source at high galactic latitude and suggested extragalactic identification

THE discovery of a number of bright, transient X-ray sources in the Milky Way has been a feature of the first year in orbit of the Ariel V satellite (see, for example, refs 1-4). It is becoming clear from the all-sky data obtained by the Ariel V Sky Survey Experiment (SSE) that at least two distinct classes of (fainter) high latitude transient X-ray source also exist. A particularly interesting first example is reported here.

The SSE detector used in these observations has an effective area at 2-18 keV of 290 cm<sup>2</sup>, and sweeps around the satellite spin plane with a fan-beam collimator of 0.7° × 10° (FWHM), the larger dimension inclined at 65° to the plane. An X-ray source that is detected may be located along a 'line of position', determined by the instantaneous position of the collimator field of view. The width of this 'line of position' is set by the errors in the measurement and in the transfer from spacecraft to celestial coordinates and is a measure of the uncertainty in the source position. A significant improvement in the overall source location can generally be obtained by carrying out a 'cross scan', to obtain an ideally perpendicular 'line of position'. Further details of the SSE and its operation will be published elsewhere.

Our observations are summarised in Fig. 1 and record the discovery of a new X-ray source in Ursa Major, 65° from the galactic plane, in late May, 1975. No source has previously been reported in this region but an extended observation with the SSE during 1975 May 3-9 produced a 3σ signal corresponding to 1.2 Ariel counts s<sup>-1</sup> (= 3.3 Uhuru counts s<sup>-1</sup> for a Crab-like spectrum). It was not visible, at a 2σ upper limit of 1 Ariel count s<sup>-1</sup>, during 1975 May 9-18 but was present at a substantially higher intensity when the SSE again viewed this region on May 22. Continuous observation to June 1 showed the source to intensify further to a peak ~16 counts s<sup>-1</sup> on May 28 and then fade in an irregular manner over the following 4 days, while the source remained in the SSE field of view. Further extended observations in mid June, early July and again at the end of August showed the source to be still faintly visible at ~0.7 counts s<sup>-1</sup>. Since the Ariel V Sky Survey is in general deeper than any previous X-ray survey, it cannot yet be ruled out that the source has a normal X-ray intensity of ~0.7 counts s<sup>-1</sup>. If so, it should perhaps be described as highly variable rather than transient, with an order of magnitude increase over a period of 10-28 d, and an additional 'flare' lasting ~1 d, during which the intensity increased by a factor of ~2.

Fig. 1 X-ray light curve of A1103+38. Change of scale of the abscissa at MJD42559. Count-rate error bars are ±1σ. Upper limits are 2σ.

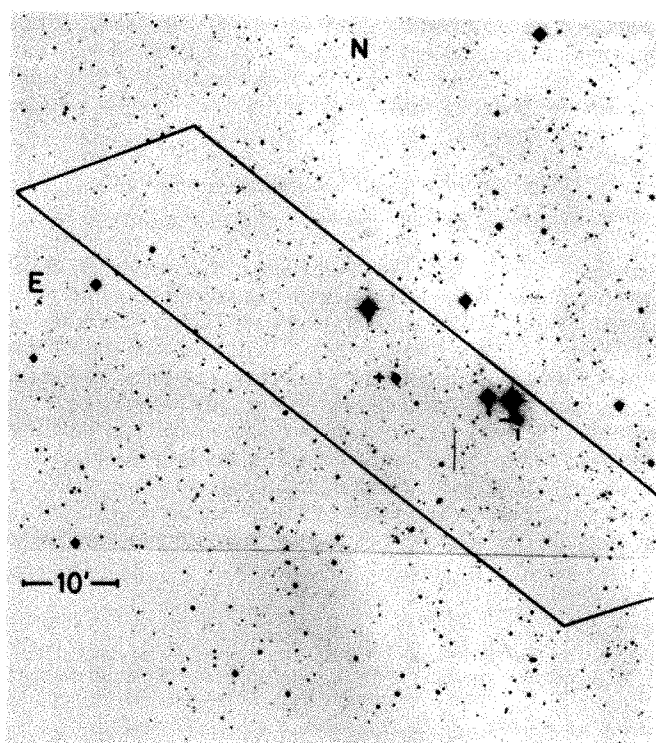
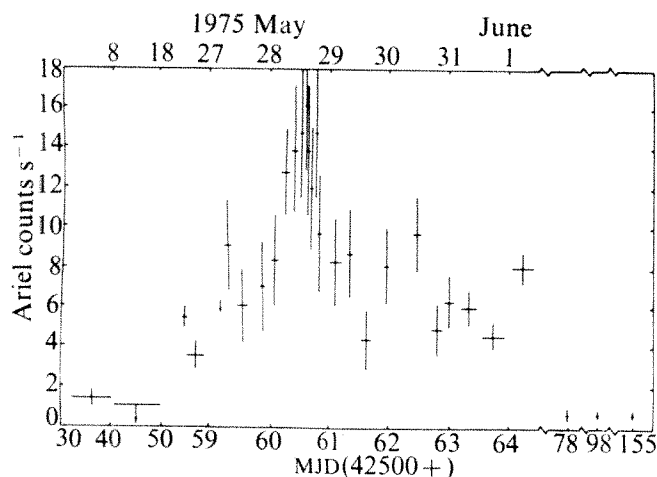


Fig. 2 90% confidence error box of A1103+38 superimposed on a composite of two adjacent, red Palomar Sky Survey prints. The suggested optical candidate MK421 (= B2 1101+38) is indicated lying 0.25° from the centre of the error box (+). The error box has coordinates:

	RA	dec.
Centroid	165.76	38.56
Corners	164.92	38.23
	165.34	38.14
	166.59	38.87
	166.17	38.97

Figure 2 is a copy of the Palomar Sky Survey plate for this region of the sky, on which has been superimposed the 90% confidence error box of the source, designated A1103+38. The narrow dimension of the box represents the line-of-position from the observations of the source near its peak brightness, and includes a statistical error of 0.03° (corresponding to a width of ±2σ, where

$$\sigma = \frac{\text{FWHM}}{\sqrt{6}} \frac{\sqrt{(2N_b + N_s)}}{N_s}$$

and  $N_b$  and  $N_s$  are the background and signal count rates) plus 0.07° representing the uncertainty in relating the spacecraft attitude to celestial coordinates. The broad (E-W) extension of the error box is from the cross scan line of position where the poorer statistics make the attitude error negligible in comparison.

A preliminary search of the error box for the optical counterpart of A1103+38 has produced three interesting candidates. Two, which are just outside the W corner of the box are IC2615 and IC2619, described in Dreyer's catalogue as faint, round, small and with a brighter middle or nucleus. From this early description it seems possible that either could be distant globular clusters located in the galactic halo (T. A. Matilsky, unpublished). The association of a class of variable, galactic sources with globular clusters has, of course, been firmly established<sup>5,6</sup>. The third, and preferred, candidate lies within the error box of A1103+38 and only 0.25° from the centroid position. This is the radio source B2 1101+38 (marked in Fig. 2), which has been identified<sup>7</sup> with the faint elliptical

galaxy MK421, distinguished by an unusually bright and optically variable nucleus. Ulrich has suggested<sup>8</sup> that this object might be of the BL Lacertae type based on the featureless continuum spectrum. Although later studies<sup>9</sup> have revealed faint absorption lines from the surrounding galaxy (yielding a redshift of 0.0308), a search of the Harvard archival plates<sup>10</sup> has shown rapid variability of an amplitude greater than for all other BL Lac-type objects, and exceeded only by two QSOs. The proposed identification with A1103+38 is necessarily subjective at this stage and depends mainly on the very unusual nature of MK421 and, in particular, the time scale of its optical variability ( $> 1$  mag over several days, ref. 12). The rarity of this type of high latitude X-ray source is also important. To date, it seems unique in its persistence. Several others detected by Ariel V last only hours (B.A.C. *et al.*, unpublished). On the other hand, several of the unidentified high latitude sources in the 3U catalogue are not seen by Ariel V, indicating variability of at least a factor of 5. It seems likely that objects of the BL Lac type are an intermediate class lying between galaxies and QSOs and it is clearly of considerable interest to establish whether, like QSOs, the active nuclei of BL Lac objects are powerful X-ray emitters. With several X-ray satellites now in orbit it should be possible to make more extended observations, coordinated with ground based studies at radio, optical and infrared wavelengths.

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## Mass determination for the X-ray binary system Vela X-1

THE 6.9 mag B0.5 Ib supergiant HD77581 has been identified as the optical counterpart of the X-ray eclipsing binary system 3U0900-40 (Vela X-1)<sup>1,2</sup>, which eclipses with a period of  $8.95 \pm 0.02$  d. The discovery of regular X-ray pulses in Vela X-1 has also been reported<sup>3</sup>, with a mean pulse period of 282.9 s, modulated because of the radial velocity variation of the X-ray pulsar in its orbital motion. This makes Vela X-1 the third X-ray binary system in which the orbits of both the optical and the X-ray component can be studied, and the following orbital parameters have been derived<sup>4</sup>,  $e_x = 0.15 \pm 0.05$ ,  $\omega_x = 157^\circ \pm 24^\circ$  and  $K_x = 268 \pm 12$  km s<sup>-1</sup>. Analyses of the light curve of HD77581 have shown that the heating effect is too small to be detected<sup>5</sup>. Furthermore, the star is bright and the spectral lines are not very broad. This means that here the first relatively accurate direct mass determination of both the X-ray and the early-type supergiant components of an eclipsing X-ray binary becomes possible.

Earlier studies of the radial velocity variation of HD77581 have given contradictory results<sup>6,7</sup>. For the semi-amplitude  $K$  of the orbit, values between 19 and 40 km s<sup>-1</sup> and for the

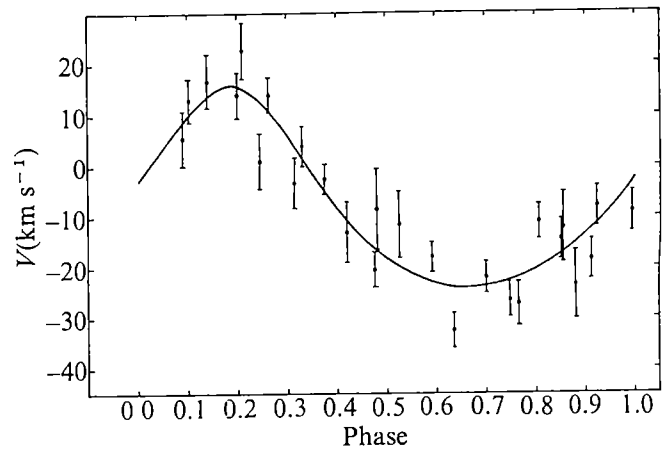


Fig. 1 Radial velocity curve of HD77581 = Vela X-1 for a period of 8.966 d. Phase zero corresponds to mid-eclipse time. The points denote mean values of the measurements of lines of He I and heavier ions. Mean errors per plate are indicated by the length of the vertical bars.

eccentricity from 0.00 to 0.54 have been found. According to Wallerstein<sup>7</sup>, the radial velocity data of HD77581 do not allow a consistent solution of the orbit, because of mass transfer in the system. Here we show that a consistent solution can be obtained, however, provided that the lines of hydrogen—expected to be most sensitive to gas motions in the system—are excluded from the analysis. Our analysis of the radial velocity variations of HD77581 is based on 26 coude spectrograms, obtained with the 152-cm telescope of the European Southern Observatory, La Silla, Chile, in 4 observing runs between April 1973 and June 1975. The spectra were taken on Il-a-0 emulsion, over wavelengths from 3,600–4,950 Å. The dispersion of the plates is 12 Å mm<sup>-1</sup> or 20 Å mm<sup>-1</sup>. The plates obtained in the first observing period<sup>8</sup> have been remeasured independently for use in this analysis (Fig. 1).

The spectra were measured for line positions with the Grant comparator of the Kapteyn Astronomical Laboratory of the University of Groningen. All absorption features visible in the spectrum were measured, without selecting beforehand a particular set of lines. Some very weak lines were thus missed on some plates, but on the other hand, especially for the weak lines, it was considered an advantage to measure without a

Table 1 Journal of observations

Plate no.	JD2440000+	phase*	$v_{rad}$ (km s <sup>-1</sup> )	m.e.	O—C
G3834	1773.626	0.480	-8.64	7.79	+ 8.00
G3842	1774.608	0.590	-18.02	3.18	+ 4.77
G3848	1775.582	0.699	-22.03	3.22	+ 1.76
G3858	1776.583	0.810	-11.00	3.36	+ 9.25
G3866	1777.613	0.925	-7.72	4.14	+ 3.93
G3879	1779.535	0.139	+16.74	5.24	+ 3.11
G3896	1780.632	0.262	+13.85	3.35	+ 2.68
G3909	1781.631	0.373	-2.37	3.11	+ 2.18
G3923	1782.556	0.476	-20.48	3.51	- 4.18
F1684	2169.522	0.636	-32.65	3.33	- 8.88
F1694	2170.522	0.747	-26.57	3.46	- 3.76
F1702	2171.477	0.854	-14.37	4.06	+ 3.25
F1709	2173.593	0.090	+ 5.68	5.52	- 2.80
F1717	2174.581	0.200	+14.16	4.58	- 1.39
F1727	2175.594	0.313	-3.27	4.94	- 7.56
F1739	2176.559	0.421	-13.00	6.06	- 2.36
F1750	2177.492	0.525	-11.60	6.31	+ 8.22
F1765	2180.463	0.856	-12.23	7.00	+ 5.23
G6480	2439.647	0.763	-27.27	4.80	- 4.98
G6491	2440.710	0.882	-23.62	6.72	- 8.12
G6497	2441.734	0.996	-8.92	3.82	- 5.24
G6505	2442.684	0.102	+12.98	4.23	+ 3.03
G6511	2443.658	0.211	+22.75	5.54	+ 7.51
G6519	2444.729	0.330	+ 3.80	3.72	+ 2.08
F3116	2560.509	0.243	+1.18	5.44	-11.90
F3124	2566.531	0.915	-18.34	4.19	- 5.73

\* Phase zero corresponds to mid-eclipse time JD2441446.54 +  $n \times 8.966$  d.



Table 2 Orbital elements for HD77581 = Vela X-1

	Mean values for He I and heavier elements	He I	Heavier ions	X-ray pulsar
$P(d)$	8.966	8.966	8.966	8.96
$V_0(km\ s^{-1})$	$-7.97 \pm 0.82$	$-8.45 \pm 0.64$	$-7.16 \pm 1.06$	
$K(km\ s^{-1})$	$19.81 \pm 1.19$	$20.54 \pm 0.99$	$21.19 \pm 1.42$	$268 \pm 12$
$e$	$0.20 \pm 0.06$	$0.23 \pm 0.04$	$0.22 \pm 0.07$	$0.15 \pm 0.05$
$\omega$ (deg)	$10 \pm 17$	$18 \pm 11$	$2 \pm 19$	$157 \pm 24$
$a \sin(i)$ (km)	$2.4 \pm 0.2 \times 10^6$	$2.5 \pm 0.1 \times 10^6$	$2.6 \pm 0.2 \times 10^6$	$3.27 \pm 0.12 \times 10^7$

predetermined expectation of where lines should be found. Lines of H I, He I, O II, N III, N II, Si III and Si IV were present in at least half of the spectra. From the measurements of the radial velocity of the interstellar Ca II K-line, we found  $\bar{v}_{Ca II} = 16.1 \pm 0.6$  (m.e.) and  $13.7 \pm 1.0$  km s<sup>-1</sup> for the 12 Å mm<sup>-1</sup> and the 20 Å mm<sup>-1</sup> spectra, respectively. To increase the homogeneity of the data we reduced the measurements of the 20 Å mm<sup>-1</sup> plates to the 12 Å mm<sup>-1</sup> system, by applying the correction of 2.4 km s<sup>-1</sup>.

To get an impression of the internal accuracy of these measurements five plates were measured twice. The differences in the mean velocity obtained from two such measurements of one plate vary between 0.3 and 4.2 km s<sup>-1</sup>; the s.d.m. per line for one plate varies from 8.0–11.5 km s<sup>-1</sup>.

In the analysis we used mean values of the radial velocity as obtained from the He I lines and from the lines of heavier ions. These average radial velocities are given in Table 1. A full table of all individual line radial velocity measurements will be published elsewhere.

With the computer program 'Orbit', based on a program of Wolfe *et al.*<sup>8</sup>, the best fitting radial-velocity curve through the points was computed. This was done for all lines of He I and the heavier ions together. Also separate solutions for the He I lines and the heavier ion lines were made. The radial velocity measurements were weighted according to  $w_i = 1/\sigma_i^2$ , where  $\sigma_i$  is the s.d.m. of the measurements. The average orbital period  $P$  determined in these solutions is  $8.966 \pm 0.005$  d, which agrees well with Hutchings' result<sup>6</sup> ( $P = 8.966 \pm 0.001$  d), based on radial velocity determinations by several observers, over a time interval of 17 yr. We therefore subsequently used this period as a fixed parameter in our calculations. The orbital parameters derived from all lines together—except the hydrogen lines—are given in Table 2. The errors quoted in Table 2 are 1σ (68% confidence) limits. The separate solutions for He I and the heavier ions are in good agreement with the mean solution (see Table 2), and give good agreement with the X-ray pulsar data, which for convenience are also given in Table 2. In particular the values of the eccentricity and the angle of periastron, which should be 180° apart for the optical and the X-ray solution, agree well (within the quoted accuracy intervals).

In our opinion this agreement lends confidence to the orbital character of the optical radial velocity variations; the non-orbital (gas streams, stellar wind fluctuations) component does not seem to have much effect on the results from He I and heavier elements. Using  $a_{opt} \sin i = 2.5 \pm 0.2 \cdot 10^6$  (1σ) as a compromise value, we find the following values for the system parameters

$$\begin{aligned} \text{mass ratio: } \mathcal{M}_{opt}/\mathcal{M}_X &= (a_X/a_{opt}) = 13.1 \pm 1.65 \\ \text{total mass: } (\mathcal{M}_{opt} + \mathcal{M}_X) \sin^3(i) &= 21.6 \pm 2.2 \mathcal{M}_\odot \end{aligned}$$

$\mathcal{M}_X \sin^3(i) = 1.52 \pm 0.22 \mathcal{M}_\odot$  and  $\mathcal{M}_{opt} \sin^3(i) = 20.0 \pm 2.1 \mathcal{M}_\odot$ . From a detailed analysis of the optical-light variations, Avni and Bahcall<sup>9</sup> have found that the inclination  $i$  should be  $> 74^\circ$  to get a consistent picture of both the observed light curve and the duration of the X-ray eclipse. Taking  $74^\circ$  and  $90^\circ$  as lower and upper limits of the angle of inclination we get

$$\mathcal{M}_X = 1.61 \pm 0.27 \mathcal{M}_\odot \text{ and } \mathcal{M}_{opt} = 21.2 \pm 2.6 \mathcal{M}_\odot.$$

All quoted errors in the mass parameters are 90% confidence limits. This result shows that the compact component is very probably too heavy to be a white dwarf.

If it is a white dwarf, its evolutionary history implies that it should consist mainly of carbon and oxygen<sup>10</sup>; the upper mass limit<sup>11</sup> for such white dwarfs is  $\sim 1.4 \mathcal{M}_\odot$ . Its most probable mass of  $1.61 \mathcal{M}_\odot$  is just consistent with the presently allowed theoretical masses of neutron stars<sup>12</sup> ( $\mathcal{M} \leq 1.6 \mathcal{M}_\odot$ ).

The mass determination of the supergiant allows a test of the theoretically computed evolution of massive stars, through a comparison with theoretical evolutionary tracks. The luminosity of HD77581 can be inferred in two ways. The spectral type and luminosity class (B0.5 Ib) provide, in principle, the absolute magnitude  $M_v$ , bolometric correction BC and effective temperature  $T_{eff}$ . Using the luminosity calibration of Blaauw<sup>13</sup> or Keenan<sup>14</sup> we derive  $M_v = -5.9 \pm 0.4$  mag. For the bolometric correction, values between 2.4 and 2.6 mag have been given<sup>15,16</sup>. This gives  $M_{bol} = -8.4 \pm 0.5$  mag. For the orbital parameters given here and by Rappaport and McClintock<sup>4</sup>, and assuming a minimum observed eclipse angle of  $34^\circ$ , Avni and Bahcall<sup>9</sup> derive for the radius of HD77581  $R = 30 R_\odot$ . For the effective temperature, values ranging from 22,000 K (ref. 17)–29,000 K (ref. 18) have been given for early B-type supergiants. Adopting  $T_{eff} = 25,000 \pm 4,000$  K we find  $M_{bol} = -9.0 \pm 0.75$  mag. From a comparison with evolutionary tracks<sup>19,20</sup> we then find the following values of the evolutionary mass:  $\mathcal{M}/\mathcal{M}_\odot = 22 \pm 7$  and  $30 \pm 10$ , respectively; these values, although not very accurate, are consistent with the presently determined value of  $21.2 \mathcal{M}_\odot \pm 2.4 \mathcal{M}_\odot$ . Therefore, the early-type supergiant in this X-ray binary system does not seem to be particularly under-massive for its spectral type (as has been suggested<sup>21</sup> for Cygnus X-1).

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## Implications from the absence of a $^{41}\text{K}$ anomaly in an Allende inclusion

CLAYTON<sup>1</sup> has elaborated on his unorthodox interpretation<sup>2</sup> of the occurrence in meteorites of the decay products of the extinct radionuclides  $^{129}\text{I}$  and  $^{244}\text{Pu}$ . He has suggested that these radioactive species did not decay in the meteorites but rather in interstellar grains which, on the formation of the meteorites, were incorporated without much alteration. Thus, the concentration in the meteorites of the decay products does not reflect the time of meteorite formation relative to the cessation of nucleosynthesis but is essentially governed by the fraction of interstellar grains mixed with the meteoritic matter proper. If that is true, anomalies will occur in the isotopic composition of numerous elements. The decisive criterion of whether an over-abundance of a given isotope can be expected is the production yield during nucleosynthesis of a radioactive precursor; the half life of that precursor is of only minor importance.

Among the possible anomalies mentioned by Clayton is an excess of  $^{41}\text{K}$ . According to Woosley *et al.*<sup>3</sup> this nuclide is synthesised predominantly as  $^{41}\text{Ca}$ , its radioactive progenitor. Therefore, in an early condensate from the solar nebula, enriched in Ca relative to K,  $^{41}\text{K}$  should be over-abundant provided that no homogenisation of potassium has occurred later on. We wish to report on a potassium isotopic analysis performed on an inclusion from the Allende carbonaceous chondrite.

For three reasons Allende seems to be a promising meteorite. First, it is almost unanimously agreed that it contains inclusions of an early primary condensate from the solar nebula (for an alternative view see ref. 4). Second, variations in the isotopic composition of oxygen<sup>5</sup> and magnesium<sup>6,7</sup> in the inclusions show that a disequilibrium distribution has been frozen in and indicate that those two elements at least have not been homogenised isotopically during the subsequent history of the meteorite. Third, almost by definition, Ca, being a refractory element, is enriched in such inclusions, whereas potassium is depleted. The resultant Ca/K ratio is thus exceedingly high, which results in a high sensitivity for the detection of a possible contribution from  $^{41}\text{Ca}$  to  $^{41}\text{K}$ .

We conducted our experiment with a 10.6-mg sample of a white chondrule previously analysed by thermal neutron activation analysis<sup>8</sup>. (Although in the course of this analysis the sample was exposed to a dose of  $1.5 \times 10^{18}$  thermal neutrons  $\text{cm}^{-2}$ , it is of no consequence to the investigation.) After the sample was dissolved the potassium was separated on Dowex 50 WX8 and analysed by mass spectrometry. Details of the chemical procedure and the isotopic analysis have been given elsewhere<sup>9</sup>. Results pertinent to this discussion are—K-content:  $210 \pm 20$  p.p.m.; Ca/K:  $860 \pm 100$  (see ref. 8); potassium blank:  $3.4 \pm 0.5$   $\mu\text{g}$ ; measured ratios:  $^{39}\text{K}/^{41}\text{K} = 13.997 \pm 0.015$ ;  $^{41}\text{K}/^{40}\text{K} = 575.3 \pm 3.7$ .

As we made no attempt to determine absolute isotope abundances the measured ratios have to be normalised. We therefore analysed a terrestrial potassium standard. Using the NIER (ref. 10) value of  $^{39}\text{K}/^{41}\text{K} = 13.47$ , and assuming the mass discrimination between neighbouring isotopes to be constant, we obtained  $^{41}\text{K}/^{40}\text{K} = 584.87 \pm 0.8$  (mean value of

16 runs) which is in good agreement with that of 584.89 given by Burnett *et al.*<sup>11</sup> who normalised to the same  $^{39}\text{K}/^{41}\text{K}$  ratio.

Because it is necessary to normalise the measured ratios an unequivocal analysis of the Allende data is only possible if any potential anomaly occurs in the abundance of one isotope only. Thus, assuming that in the Allende inclusion the  $^{39}\text{K}/^{40}\text{K}$  ratio is identical to the terrestrial value our result shows that the enrichment in the abundance of  $^{41}\text{K}$  is  $\delta^{41}\text{K} = (0.55 \pm 1.3)\%$ . If the  $^{39}\text{K}/^{41}\text{K}$  ratios are the same in both cases  $\delta^{40}\text{K} = -(0.3 \pm 0.7)\%$ . It is worth mentioning that, within reasonable limits, the  $\delta$  values are rather insensitive to the ratios used for normalisation. A change in the choice for the terrestrial  $^{39}\text{K}/^{41}\text{K}$  by  $\pm 5\%$  results in even smaller, but comparable, relative changes of the  $\delta$  values.

Within the stated errors the isotopic composition of potassium from the Allende inclusion is indistinguishable from that of terrestrial potassium. This constrains certain interesting parameters. The absolute excess in the abundance of  $^{41}\text{K}$  is  $\Delta^{41}\text{K} = [\text{K}] \times \text{H} (^{41}\text{K}) \times \delta^{41}\text{K}$  where  $[\text{K}]$  is the potassium content (including blank) and  $\text{H} (^{41}\text{K})$  the isotopic abundance of  $^{41}\text{K}$ . This is equal to the amount of  $^{41}\text{Ca}$  ( $= ^{41}\text{Ca}/^{40}\text{Ca} \times [\text{Ca}] \times \text{H} (^{40}\text{Ca}) \times f$ ) which decayed in the sample. Assuming<sup>1,2</sup> that the condensation of Ca took place quickly after the synthesis of  $^{41}\text{Ca}$ , that is, that no correction has to be applied for its decay (half life  $1.3 \times 10^5$  yr) before condensation (see refs 1 and 2),  $f$  denotes the fraction of presolar Ca in the sample. The data presented here show that, on the  $3\sigma$  level ( $\delta^{41}\text{K} \leq 5\%$ ),  $^{41}\text{Ca}/^{40}\text{Ca} \cdot f \leq 10^{-5}$ . Clayton<sup>2</sup> reasons that for refractory elements like Ca,  $f \geq 0.1$ . Thus, in the presolar grains at the time of their formation the  $^{41}\text{Ca}/^{40}\text{Ca}$  ratio must have been  $\leq 10^{-4}$ . According to Peters *et al.*<sup>12</sup>, however, the production ratio in explosive carbon, oxygen, or silicon burning is about a factor of 30 higher ( $1/320$ ).

Of the numerous possibilities which could account for the discrepancy between our experimental result and theoretical prediction a few are worth listing.

- The cross sections relevant to the  $^{41}\text{Ca}/^{40}\text{Ca}$  production ratio are not known; they could be considerably lower than assumed<sup>12</sup>.

- Even for refractory elements the fraction,  $f$ , of presolar grains in the meteoritic matter may be lower than 0.1, although it is doubtful that with a 30-fold reduction the  $^{244}\text{Pu}$  data are still reconcilable with the model.

- Radioactive decay of  $^{41}\text{Ca}$  between cessation of nucleosynthesis and condensation of the grains may not be negligible after all; a free decay interval of about 0.5 Myr would be sufficient. In that case the number of possible isotope anomalies mentioned by Clayton<sup>1</sup> will be drastically reduced, as  $^{41}\text{Ca}$  has one of the longest half lives among the radioactive progenitors.

- Homogenisation of potassium and/or calcium may have occurred in the sample analysed. This would have wiped out, or drastically reduced, any anomaly which may have existed. A late addition of potassium of normal isotopic composition is not sufficient, however.

- The isotope anomaly in potassium may not be restricted to one isotope only, as has had to be assumed here for an unambiguous interpretation. Only a determination of the absolute abundance ratios without normalisation will allow that possibility to be checked.

As we realise that it would be helpful to know whether our sample shows anomalies in the isotopic composition of other elements, such as Mg, such measurements are planned.

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## Devonian palaeogeography of the Orcadian Basin and the Great Glen Fault

STORETVEDT<sup>1</sup> has argued that Devonian palaeomagnetic results from Norway and northern Scotland indicate a post-Devonian sinistral displacement along the Great Glen Fault of some 200–300 km. This has been objected to on geological grounds by Mykura<sup>2</sup> and on palaeomagnetic grounds by

Turner *et al.*<sup>3</sup>. Here we present a simplistic Devonian palaeogeographical model of the Orcadian Basin based on observation of onshore sediments. We believe the model is compatible with only minor dextral displacement along the Great Glen Fault in post-Devonian times.

A variety of modes of displacement have been put forward for the Great Glen Fault. Some are based on pre-Devonian evidence<sup>4,5</sup> and are not considered relevant to our argument. We consider the following three:

First, sinistral movements of 200–300 km (ref. 1) or ~ 500 km (ref. 6).

Second, dextral movements of:

- (1) ~ 30 km on the Great Glen Fault s.s.<sup>7</sup>
- (2) ~ 65–80 km on the Walls Boundary Fault in Shetland<sup>8–11</sup>
- (3) a considerable movement on the Melby Fault in Shetland<sup>12</sup>

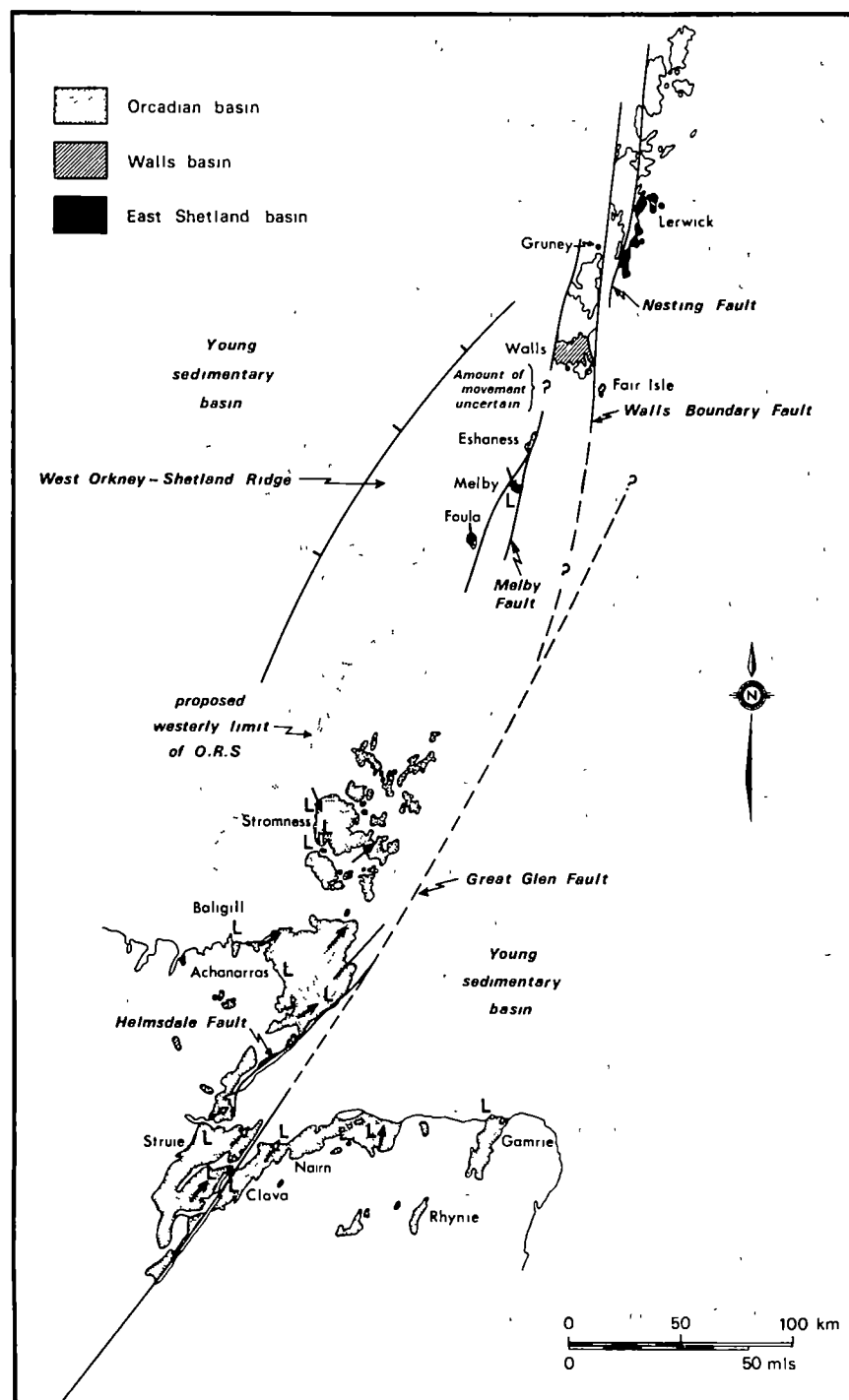


Fig. 1 Reconstruction of Devonian palaeogeography in the Orcadian Basin. Sources as described in the text, plus Watts<sup>21</sup>. L is the Achanarras limestone and equivalents. Open arrows show Upper ORS palaeocurrents (RA) and closed arrows show Middle ORS palaeocurrents (Orkney from Mykura<sup>12</sup>, elsewhere from R.N.D.).

- (4) ~ 50 km on the Helmsdale Fault<sup>8</sup>
- (5) a substantial movement on the Latheron Fault in south eastern Caithness<sup>13</sup>.

Third, normal movements:

- (1) Post-Jurassic movement of the Great Glen Fault s.s. associated with development of the Moray Firth Basin<sup>14</sup>
- (2) Post Jurassic movement of the Walls Boundary Fault associated with basin development to the south west of Shetland<sup>15</sup>.

Of the three distinct basins of ORS sedimentation in Shetland, considered to be juxtaposed by dextral faulting<sup>1,12</sup> only the westernmost had tangible connection with the Orcadian Basin to the south (Fig 1), that is, the similarity of the fauna of the Melby Fish Beds with those at Sandwick (Orkney) and Achanarras (Caithness). Mykura<sup>12</sup> has shown that a post-Devonian sinistral movement of 200–300 km is unlikely, as it places the dissimilar Orcadian and East Shetland sequences in juxtaposition. A movement of ~ 500 km certainly resolves the contradiction but has the great disadvantage of placing the ORS sediments of the southern Basin to the east of the Great Glen Fault system, in a position now occupied by the coast of Galway, western Ireland. Such a position is unlikely as the ORS sediments of the Orcadian Basin on both sides of the Great Glen Fault are very similar in character and show an identical history of development which is unique in the British Isles, and comprises:

- (1) Lower ORS (Upper Seigennian and Emsian) sedimentation of mainly alluvial character (ref. 16 and A. Collins, R.N.D. and R.A., unpublished).
- (2) Unconformity associated with mild earth movements.
- (3) Middle ORS (Eifelian and Givetian) sedimentation of mixed lacustrine and alluvial character.
- (4) Unconformity associated with earth movement and volcanism in Orkney.
- (5) Upper ORS (Frasnian and Lower Fammenian) sedimentation of alluvial character<sup>15</sup>.

During the extensive Middle ORS deposition faunal evidence indicates a most intimate connection between areas on either side of the Great Glen Fault system. Specifically the Achanarras Fish Bed fauna is represented in the southern part of the Orcadian Basin at, for example, Edderton and Cromarty to the west of the fault system and Clava, Nairn, Lethen Bar, Tynet Burn and Gamrie to the east (Fig. 1). The age equivalence of these beds is based on vertebrate faunas<sup>17,18</sup> and also on palynological data<sup>19</sup>. Furthermore, the zonal faunal assemblages recognised in the Caithness Flagstones<sup>13</sup> occur to the east of the Great Glen Fault system in the Nairn-Clava region in identical stratigraphic order<sup>20</sup>. At least 18 species of fish are, in fact, common to both sides of the fault system.

The existing outcrops of the Middle ORS are remnants of the western and southern margins of an elliptical basin elongated in a north-east-south-west direction (Fig 1). The original focus of sedimentation in this basin (as exposed) was in the Caithness-Orkney area where a distinctive, dominantly lacustrine, facies developed. Subsequently overlap to the north, west, south and south-east took place. In these latter areas alluvial deposits are more in evidence. Throughout the basin a rhythmic pattern of sedimentation was produced by variation in the level of Orcadian lakes. During high lake levels, when lake waters lapped directly against the underlying Caledonian basement, a distinctive marginal limestone facies was deposited<sup>21,22</sup>. This facies has recently been found on the south shore of the Moray Firth and confirms the overlap discussed above. Palaeocurrent vectors in both the Middle and Upper ORS support deposition of the sediments within a single basin (Fig. 1).

Within the palaeogeographic framework a relatively modest post-Devonian dextral shift of 30 km (ref. 7) is

acceptable and indeed produces a better 'fit' of Middle ORS outcrops in the Inverness area. There would seem to be no geological evidence to support large scale post-Devonian sinistral movement along the Great Glen Fault system although a variety of motions could have occurred in pre-Devonian times.

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## Kinetic processes and thermal history of slowly cooling solids

In solids, the rates of kinetic processes such as volume diffusion and order-disorder transitions are extremely sensitive to temperature. At room temperatures they are so slow that crystalline solids normally preserve a substantial degree of thermodynamic disequilibrium over very long periods ( $> 10^9$  yr in some cases). At sufficiently high temperatures, on the other hand, some processes go so fast that a continuously varying state of equilibrium may be maintained as the temperature changes. When a solid cools steadily a transition occurs from the one state (continuous equilibrium) to the other (false equilibrium) in the neighbourhood of the closure temperature, which is defined below. Here I present a simple quantitative argument relating closure temperature to the cooling rate and kinetic parameters of a given kinetic system (see refs 1–6 for related work).

Closure temperature<sup>1</sup> is defined in Fig. 1. Curve I represents the equilibrium value of some chemical parameter  $x$ , and curve II its kinetically-controlled value. At high temperatures the two curves coincide. When the temperature decreases sufficiently the kinetically-controlled value begins to change more slowly than the equilibrium value, and the curves diverge. Eventually  $x$  reaches a constant, non-equilibrium value, corresponding to some earlier temperature (see Fig. 1), which is the closure temperature  $T_c$ . Slower rates of cooling allow more time for re-equilibration, and therefore lead to lower values of  $T_c$ .

Analytic derivations of closure temperature have been presented for some simple first-order kinetic systems<sup>1</sup>. A graphical solution has been attempted for one second-order system<sup>3</sup>. Here a physical argument is given which is applicable, in an approximate way, to all kinetic systems which obey the Arrhenius law. The argument compares a cooling time constant



with estimated isothermal reaction times at various temperatures.

For Arrhenius processes the reaction rate coefficient  $k(T)$  at absolute temperature  $T$  is given by

$$k(T) = k_0 \exp(-E/RT) \quad (1)$$

$E$  is the activation energy and  $k_0$  the frequency factor for the process, and  $R$  is the gas constant. For a slowly cooling system

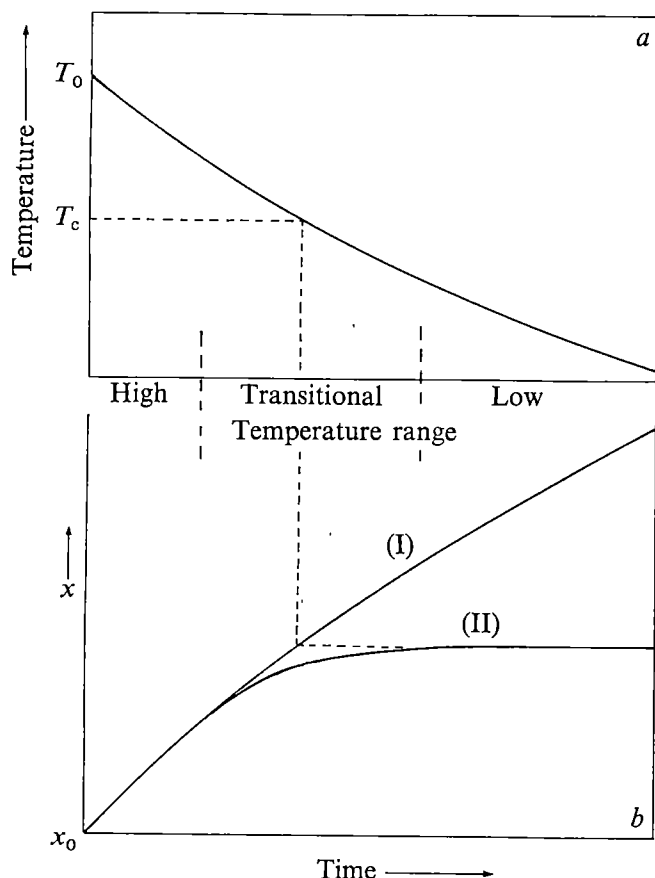


Fig. 1 Kinetically controlled closure in cooling solids. *a*, Temperature against time; *b*, parameter  $x$  against time. (I), Equilibrium value of  $x$ ; (II), actual value of  $x$ .

$k$  diminishes roughly exponentially with time. If we make the approximation

$$T^{-1} = T_0^{-1} + \text{constant} \times t \quad (2)$$

Multiplying by  $E/R$  we absorb the above constant in the cooling time constant  $\tau$

$$E/RT = E/RT_0 + t/\tau \quad (3)$$

Substituting in (1) we obtain

$$k(t) = k(T_0) \exp(-t/\tau) \quad (4)$$

so that  $\tau$  is the time required for  $k$  to diminish by a factor  $e$  in the neighbourhood of  $T$ . The relationship between  $\tau$  and the cooling rate  $\dot{T}$  is found by differentiating (3) to be

$$\tau = -RT^2/E\dot{T} \quad (5)$$

We can now compare  $\tau$  with the time required for the kinetic process to go, say, half-way towards completion at constant

temperature, which must be inversely related to the rate coefficient, so we can write

$$kt_{1/2} = \alpha \quad (6)$$

(For a first-order process  $\alpha = 0.693$ ; for simple diffusion processes it is smaller and depends on the initial distribution and the geometry; for second or higher-order kinetics  $\alpha$  depends to some extent on both the actual value of  $x$  and its equilibrium value.) At high temperatures the process must reach equilibrium in a time which is short compared with the time required for  $k$  to diminish significantly, so we infer that  $t_{1/2} \ll \alpha$ . At low temperatures, on the other hand  $t_{1/2} \gg \alpha$ : that is to say, the process is so slow that negligible progress is made towards equilibrium before the rate constant has diminished to even lower value. Finally, in the transitional range, where the temperature cools through the value  $T_c$ ,  $t_{1/2}$  and  $\tau$  are of the same order of magnitude. Thus from (6) we have

$$\tau k(T_c) \simeq \alpha \quad (7)$$

Substituting for  $k(T_c)$  and taking logarithms we obtain immediately

$$E/RT_c \simeq \ln(\tau k_0/\alpha) \quad (8)$$

Analytical solutions for first-order kinetics and for simple diffusion processes<sup>1</sup>, give

$$E/RT_c = \ln(A\tau k_0) \quad (9)$$

in which  $k_0$  may be replaced by  $D_0/a^2$ , the frequency factor for a diffusing component in a system with characteristic dimension  $a$ . Thus it seems that equation (9) can be generalised to more complex kinetics by setting  $A \simeq \alpha^{-1} = (kt_{1/2})^{-1}$ . Assuming the boundary condition (curve I) to be linear in  $1/T$  over the transitional temperature range, and therefore a linear function of time, the constant  $A$  is 1.8 for first-order reactions, and 8.7, 27 and 55 for diffusion out of a plane slab, cylinder or sphere<sup>1</sup> comparison with equation (6) and (8) using data from Crank<sup>7</sup> shows that, in these systems, a better approximation to  $A$  is given by

$$A = 2/kt_{1/2} = 2/\alpha \quad (10)$$

This gives values of  $A$  which are too high by 60% for a first-order reaction, but only 20% for diffusion problems.

Eliminating  $\tau$  between equations (5) and (9), and setting  $T = T_c$ , we obtain

$$E/RT_c = \ln(-Ak_0RT_c^2/E\dot{T}) \quad (11)$$

which can be solved explicitly for  $\dot{T}$ , or iteratively for  $T_c$  ( $k_0$  can be replaced by  $D_0/a^2$  when appropriate). The relationship between  $T_c$  and  $\dot{T}$  is plotted in Fig. 2 for various values of activation energy  $E$ . Because of the logarithmic character of the equation,  $T_c$  is rather insensitive to cooling rate, especially at low temperatures and high activation energies. For example, at 60 kcalorie mol<sup>-1</sup> a change in the cooling rate by a factor of ten changes  $T_c$  by only 20° near 250°C, or 80° near 1,000°C. At 10 kcalorie mol<sup>-1</sup> the corresponding changes are 100° and 400°. The corollary is that observations of closure temperature must be very precise to yield useful information about cooling history. The best that has yet been achieved is represented by a study of nickel concentration profiles in iron meteorites, in which numerical modelling of the diffusion/cooling process led to uncertainties of  $\pm 15\%$ – $\pm 50\%$  in the estimated cooling rates<sup>8</sup>. On the other hand, an approximate knowledge of cooling rates may yield rather accurate predictions of closure temperature, which may be helpful in understanding how the properties of materials depend on their cooling history (see, for example, ref. 6).

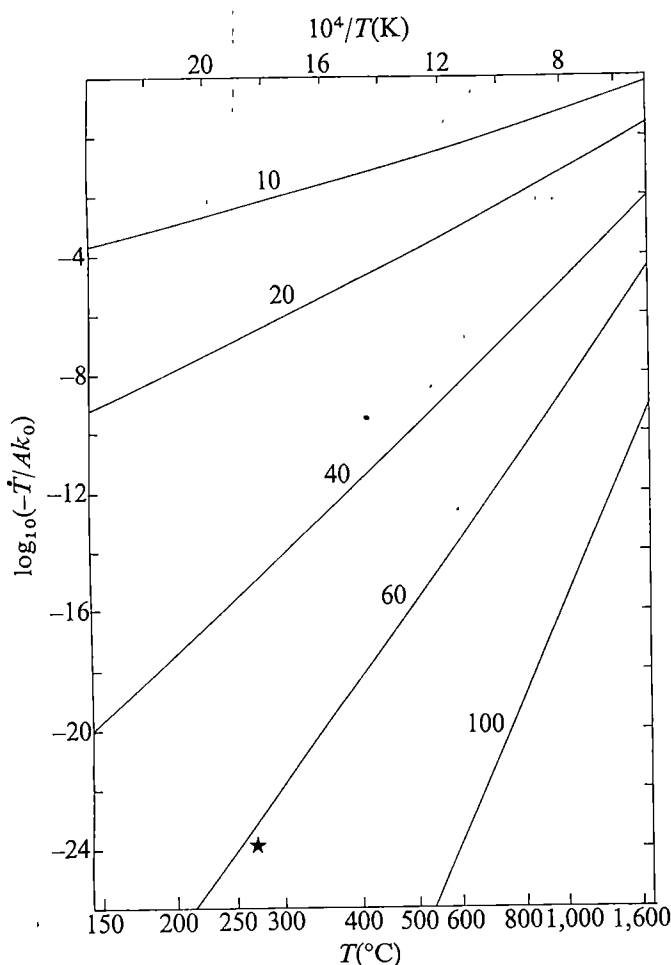


Fig. 2 Relationship between closure temperature  $T_c$ , cooling rate  $\dot{T}$ , and kinetic parameters. Figures on curves denote values of activation energy  $E$  in kcalorie  $\text{mol}^{-1}$ . Star corresponds to example discussed in text.

As an example for which analytic solutions are not yet available, we may consider the  $\text{Fe}^{2+}$ -Mg order-disorder reaction in a cooling anthophyllite<sup>3</sup>. A closure temperature of 270 °C was estimated for the untreated sample, while laboratory kinetic studies<sup>9</sup> gave  $E = 61.6$  kcalorie  $\text{mol}^{-1}$  and  $k_0 = 8.6 \times 10^{17} \text{ yr}^{-1}$ . Inspection of the kinetic data<sup>9</sup> yields an estimate for  $A$  of  $\sim 10$ , from which, using equation (11) or Fig. 2 one can calculate a cooling rate of 13 °C per million years. Seifert and Virgo<sup>3</sup> estimated, graphically, a maximum cooling rate of 100 °C per million years for this material, while typical cooling rates in Alpine metamorphic rocks are 20 °C  $\text{Myr}^{-1}$  (refs 1, 10 and 11). The principal weakness in the calculation is uncertainty in  $A$ , since for a second-order reaction,  $t_{1/2}$  depends on the initial concentrations. In such cases, the best estimate of  $A$  (other than that obtained from a full analytical or numerical solution to the problem) could perhaps be obtained by estimating  $kt_{1/2}$  for an isothermal reaction which starts with a degree of disequilibrium comparable with that expected within the transition temperature range of the cooling system.

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## Thermal conductivity of allotropic modifications of ice

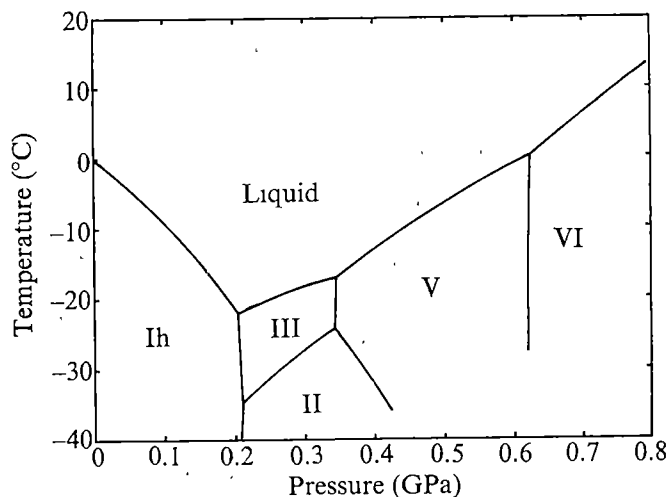
THE pressure-temperature phase diagram of  $\text{H}_2\text{O}$  comprises several solid phases, differing in their crystal structure (Fig. 1)<sup>1</sup>. Ice offers an unusual opportunity for investigating how the different modes of stacking and the slight distortions of otherwise identical molecules influence the conduction of heat. There are no previous studies of the thermal conductivity of ice, except for the phase Ih at atmospheric pressure. We report here the first measurements of the thermal conductivity of the high pressure forms II, III, V and VI. Each of these modifications has a higher density, and a lower thermal conductivity, than ordinary ice, Ih. Ice Ih seems to be the first example of an insulator which has a decreasing thermal conductivity under increasing pressure. Our findings show that all these forms of ice have a positive thermal expansion coefficient.

Our measurements were carried out using the transient hot-wire method<sup>2</sup>, in which a pulse of constant electrical power is supplied to a metal wire immersed in a medium of the substance under study. An analysis of the change of temperature over time in the wire, as monitored by its electrical resistance, enables the thermal conductivity to be determined. The pulse length used was 0.6 s. Temperature data were acquired and reduced using a programmable desk calculator. We used a nickel wire, 0.1 mm in diameter, 40 mm long between the potential leads.

The wire was situated across the diameter of a Teflon cell similar to that described by Andersson and Bäckström<sup>3</sup>, but 70 mm in diameter. This cell was filled with doubly distilled  $\text{H}_2\text{O}$ , which was degassed by boiling. The cell was subjected to pressure in a piston-cylinder apparatus, cooled by circulating Freon.

Our measurements were made over a range of pressures going up to 0.65 GPa, and at temperatures down to -35 °C. Steady-state temperature measurements were taken using three thermocouples with their junctions located at different radial distances from the axis of the vessel. Phase transitions were detected using some or all of the following observations: discontinuous changes in the thermal conductivity,  $\lambda$ ; thermocouple responses indicating latent heat; and piston displacements indicating changes of volume. Whenever the measurements showed clearly that the system was fully within a single phase

Fig. 1 Phase diagram of  $\text{H}_2\text{O}$ .



region, the temperature differences within the cell were less than 0.2 °C. We were careful to ensure that temperature excursions of the Ni wire did not raise the temperature of the ice above the range of the modification under investigation.

Our investigations of the thermal expansion coefficient,  $\alpha$ , have revealed that an approximately adiabatic pressure increase or decrease always results in a temperature change of the same sign. Using the identity  $(\partial T/\partial P)_S = T\alpha/c_p$ , we conclude that  $\alpha$  is positive in the pressure-temperature region investigated, whereas it is known to have negative values for water and, according to one report<sup>1</sup>, for ice Ih at very low temperatures.

Our results for  $\lambda$  for each of the phases of ice are given in Table 1. Data for liquid H<sub>2</sub>O (see ref. 4) are quoted for comparison. Special efforts were not made to attain an absolute degree of accuracy, but our average value for ice Ih at -25 °C and atmospheric pressure— $\lambda = 2.38 \text{ W m}^{-1} \text{ K}^{-1}$ —is within the limits of error of other determinations<sup>5,6</sup>. We measured the conductivity of the five solid phases several times and in different orders in pressure-temperature space, and the results agreed to within 1 or 2 % in each case. The temperature variation of the conductivity in phase Ih was found to conform to the  $1/T$  dependence already reported<sup>5,6</sup>. But a startling fact emerged concerning the pressure dependence. We found that the thermal conductivity decreases by 6% in going from atmospheric pressure to 0.20 GPa, whereas all the insulators previously investigated have exhibited an increase<sup>7,8</sup>. In the other phases of ice no temperature or pressure dependence could be detected over the temperature-pressure region investigated.

A rough estimate of the phonon mean free path,  $L$  (some values of  $L$  are included in Table 1), may be obtained from the well known Debye formula

$$\lambda = c_p L / 3(\nu \kappa)^{1/2}$$

In calculating  $L$  we have taken the specific volumes,  $\nu$ , from

Table 1 Thermal conductivity of H<sub>2</sub>O

Phase	$T$ (°C)	$P$ (GPa)	$\lambda$ (W m <sup>-1</sup> K <sup>-1</sup> )	$L$ (nm)
Liquid	0	0.00	0.56	0.29
Ih	-25	0.00	2.38	1.20
II	-30	0.30	1.65	
III	-25	0.25	0.98	0.43
V	-25	0.50	1.24	0.44
VI	-20	0.65	1.54	0.60

Bridgman<sup>9</sup> and used his isothermal compressibilities except for ice Ih (see ref. 10) and liquid (see ref. 11), for which the adiabatic compressibilities are known. Furthermore, we have assumed the specific heat capacity to be the same in all the solid phases. The point to be noted is that, for phases III, V and VI,  $L$  is on average about twice the value that it is for the liquid, whereas for ice Ih it is four times as large.

Bridgman<sup>9</sup> has provided data for phase Ih, showing a rapid decrease of compressibility with pressure, which would lead to a similar decrease of  $L$ . But his atmospheric pressure value for compressibility is at variance with later work<sup>12</sup>. Thus, although it would be interesting to calculate the pressure dependence of  $L$  for ice Ih, we feel this cannot yet be done reliably.

We have considered the possibility that a single crystal of ice Ih present initially near atmospheric pressure would gradually transform into a polycrystalline mass, resulting in an increased grain scattering. Measuring  $\lambda$  for decreasing as well as increasing pressure, however, we obtained perfectly reproducible results, from which we conclude that changes in grain boundary scattering cannot be invoked to explain the pressure dependence.

The phonon mean free path,  $L$ , cannot be similarly computed for ice II, since the compressibility is unknown. It should be noted, however, that the thermal conductivity of this phase is clearly larger than for other phases with a comparable density. We tentatively attribute this to the proton order in the

structure of ice II, which does not exist in the other phases. We hope to shed light on the influence of proton order by making similar measurements on D<sub>2</sub>O and its mixtures with H<sub>2</sub>O.

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## Intensity effects in the electrochemical photolysis of water at the TiO<sub>2</sub> electrode

FUJISHIMA and Honda<sup>1</sup> have described the photosensitised electrolysis of water on n-type TiO<sub>2</sub> single-crystal electrodes and proposed this reaction be used for solar-energy conversion. The process differs from conventional electrolysis since photocurrents can be generated without the application of an external potential, and thus direct conversion of light to electrical energy is possible. Since their report, several groups have investigated this system<sup>2-4</sup> but, as a consequence of the broad-band irradiation used, accurate quantum yields could not be determined. Here we report successful measurements of the variation of quantum efficiency with intensity. Our main finding is that these crystals become efficient converters if a small voltage is applied, even at intensities of 12 mW cm<sup>-2</sup>.

This is important since the degree to which sunlight can be focused without decreasing the quantum efficiency will determine whether these devices may be used economically. Intensity effects might be expected in this system since a major factor in determining the magnitude of the steady-state photocurrent is the recombination rate of the photoproduced valence-band holes and conduction-band electrons<sup>5</sup>.

To study these effects, a dual compartment electrochemical cell and single-crystal electrode were made<sup>1</sup>. A platinum cathode was used and a PAR-174 polarograph in the circuit allowed us to adjust the potential of the TiO<sub>2</sub> relative to a saturated calomel electrode (SCE). Small dark currents resulted from the application of this potential. When the TiO<sub>2</sub> electrode was illuminated, substantially higher currents were observed, and the difference between the observed current and the dark current was used to calculate the quantum efficiency of the process. The light source was a Coherent Radiation CR-8 argon ion laser emitting a doublet at 351 and 364 nm. The power incident on the electrode was varied through the use of neutral density filters, the resulting photocurrent was monitored, and the existence of a nonlinear intensity dependence was confirmed. The results depicted in Fig. 1 show marked deviation from linearity both at 0 and +2.0 V (SCE).

To obtain more data in the lower intensity range, the laser beam was directed through a beam expander on to the  $\text{TiO}_2$  surface. The resulting power loss was  $\sim 70\%$ , but with the focusing lens of the expander, it was possible to vary the size of the spot of light on the electrode surface from 0.2–2.0 cm in diameter, while maintaining constant total output power. This had the effect of varying intensity per unit area at the electrode. Three solutions having the same electrical conductivity but with different pH were used in the experiments. Results of irradiations performed at 0 V (SCE) are depicted in Fig. 2a. The dashed line represents the photocurrent expected if the quantum yield was unity and was intensity independent. Generation of the photocurrent in all three solutions becomes increasingly less efficient as intensity increases, and marked differences in behaviour are exhibited. The lowest efficiencies occur consistently for the acidic solution and the quantum yield at very low intensities is  $< 0.1$ . With the NaOH solution at intensities  $< 10 \text{ mW cm}^{-2}$ , the photocurrent is approximately linearly dependent on intensity, and the quantum yield calculated from the slope in this region is 0.7. This behaviour suggests that a pH-dependent process occurs at 0 V (SCE) at the surface of the electrode, and effects electron-hole recombination.

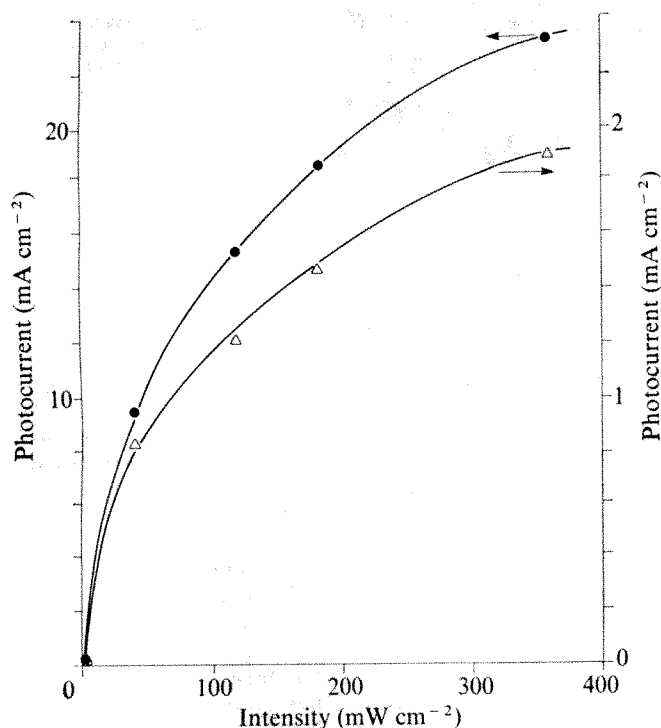


Fig. 1 The photocurrent as a function of intensity for irradiation of the  $\text{n-TiO}_2$  electrode in 0.51 N  $\text{HClO}_4$  for applied potentials of ( $\Delta$ , right axis) 0 V (SCE) and ( $\bullet$ , left axis) +2 V (SCE).

When the potential of the  $\text{TiO}_2$  electrode was raised to +1.0 V and the experiments repeated, the situation was substantially altered (Fig. 2b). Above  $12 \text{ mW cm}^{-2}$ , all three plots deviated from linearity, but even at the greatest intensity examined, the quantum yields were still high. The quantum yield of electrons at  $60 \text{ mW cm}^{-2}$  in acidic solution was 0.55 whereas in the basic solution it was 0.67. Below  $12 \text{ mW cm}^{-2}$ , the three curves are coincident and a nearly linear intensity dependence is obtained. The electron quantum yield calculated from the slope in this region is 0.85. For intensities  $< 0.5 \text{ mW cm}^{-2}$ , we have found quantum yields as high as 0.95.

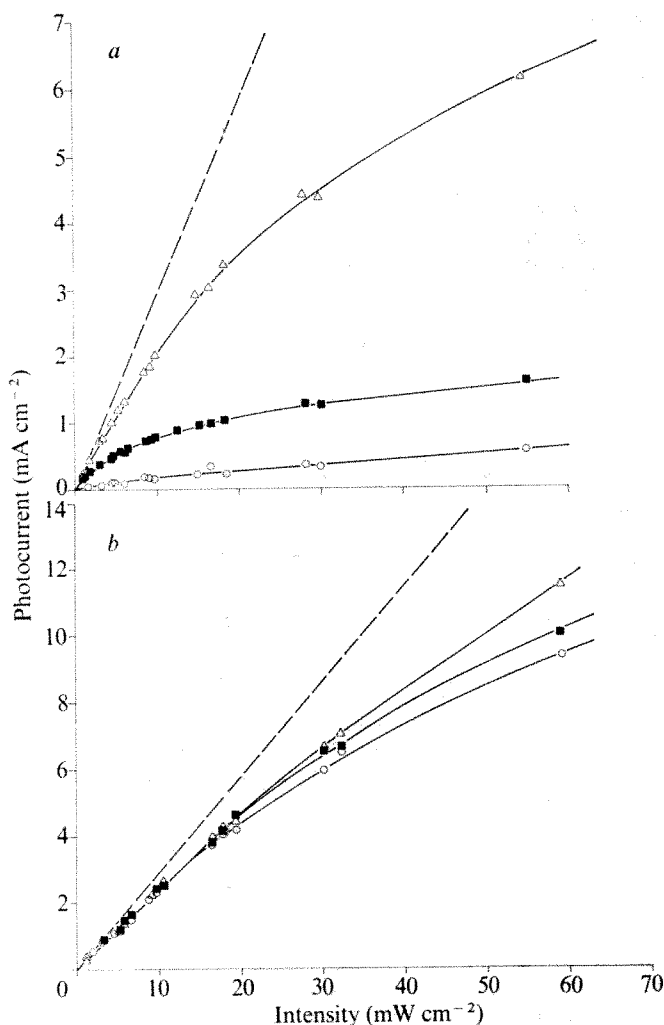


Fig. 2 The intensity dependence of the photocurrent produced on irradiation of the  $\text{n-TiO}_2$  electrode (a), at 0 V (SCE), (b) at +1 V (SCE). ( $\circ$ ) 0.2 N  $\text{H}_2\text{SO}_4$ ,  $\kappa = 41.8 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$ ; ( $\blacksquare$ ) 0.8 N  $\text{Na}_2\text{SO}_4$ ,  $\kappa = 41.3 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$ ; ( $\triangle$ ) 0.225 N NaOH,  $\kappa = 41.0 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$ .

The photoactive region for  $\text{n-TiO}_2$  is 300–400 nm (ref. 4). A total of  $\sim 2 \text{ mW cm}^{-2}$  of light in this region is available on a sunny day in our vicinity<sup>6</sup>. On the assumption that the quantum yield and intensity dependence are relatively independent of wavelength over this region, Fig. 2 can be used as an indication of the efficiency in the conditions specified. At 0 V (SCE) only  $\text{TiO}_2$  electrodes in strongly basic solutions are efficient in converting light energy into electricity. Moreover the efficiency will be dependent on the nature of the electrolyte, and if  $\text{OH}^-$  is consumed in the photolysis, it will decrease with time. At an applied potential of 1 V (SCE) however, the efficiency of the  $\text{TiO}_2$  system will be high and independent of solution pH. In addition, since the quantum yield remains relatively independent of intensity up to  $12 \text{ mW cm}^{-2}$ , some focusing of the incident radiation is allowed without a corresponding decrease in quantum efficiency.

The fact that the quantum efficiency approaches unity at low intensities for these wavelengths is significant since it suggests that further research should be directed towards broadening this wavelength range. Materials which had a photoactive region extending into the visible could utilise a larger proportion of the incident solar radiation.

Electrochemical oxidation of titanium metal to form an oxide layer<sup>7</sup>, and chemical vapour deposition of volatile titanium compounds to produce a polycrystalline  $\text{TiO}_2$  layer on suitable substrates<sup>2</sup>, have both been proposed as



cheaper alternatives to single crystal electrodes. The intensity dependence of the photocurrent at these electrodes can be expected to have a major role in evaluating their performance relative to the single crystal electrodes.

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## Effect of nitrogen-containing plasmas on stability, NO formation and sooting of flames

WE have been developing methods for burning leaner fuel/air mixtures at faster rates and with less pollution than is achieved in conventional systems<sup>1-3</sup>. Mixtures of heat contents much below those corresponding to normal limits of flammability have been burned successfully at low temperatures using heat recirculation<sup>4,5</sup>. At temperatures much above the melting point of heat exchangers large increases in burning rates for a small input of electricity were achieved when radicals from plasma jets were injected, using a magnetically rotated arc to achieve rapid mixing<sup>6</sup>. We now report on further effects which can be induced using even a much smaller plasma jet (of about the size of a conventional sparking plug), the addition of much smaller proportions of electrical energy (~ 4% of the chemical throughput) and the absence of any magnetic field.

In an effort to approach practical conditions more closely, the combustion chamber was a model of a gas turbine flame tube provided by Ricardo & Co. Ltd, consisting of a cylindrical outer chamber (15 × 60 cm) fitted with a Perspex end-viewing window. The fuel is injected through a 'Sonicore' nozzle into a Pyrex mixing tube, where the combustion air from a centrifugal blower flows at velocities much in excess of those used in

Fig. 1 Flame stabilised by nitrogen-containing plasma.

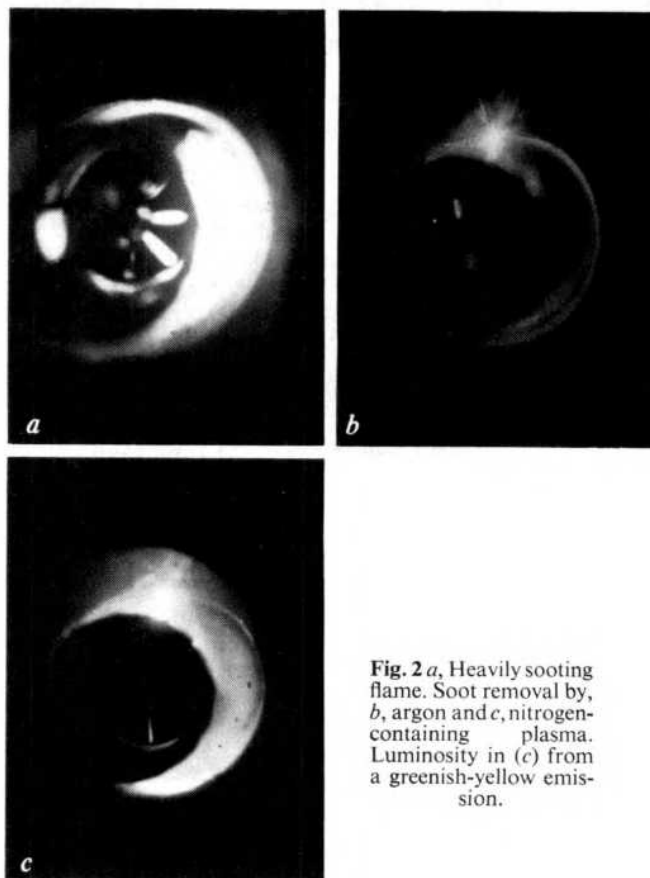
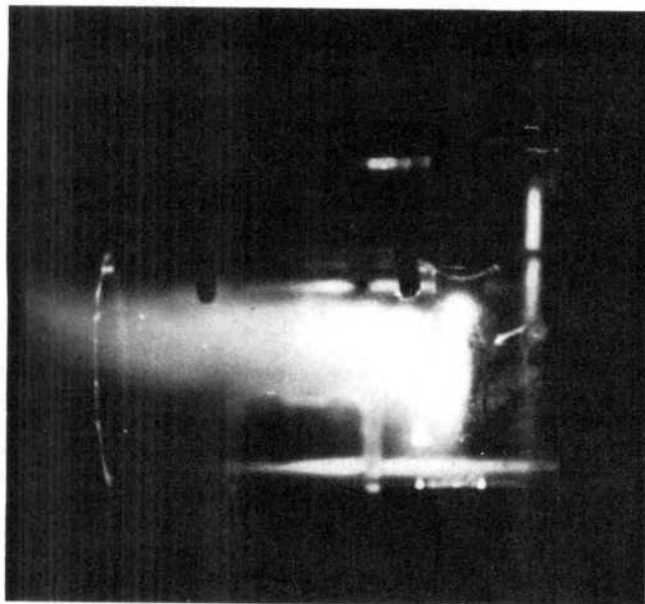


Fig. 2 a, Heavily sooting flame. Soot removal by, b, argon and c, nitrogen-containing plasma. Luminosity in (c) from a greenish-yellow emission.

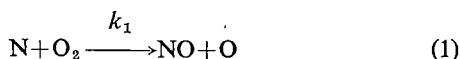
previous work. Swirl vanes upstream of the nozzle aid mixing (these are visible in Fig. 2a-c).

To allow comparison with earlier work, nitrogen-containing plasmas were used (these had been found<sup>6</sup> to give marginally the best results) and were compared with 'inert' plasmas of argon alone. Once again, very large increases in stability were observed, to the point where flames could be maintained in mixtures so lean and flow velocities so high that normal flame stabilisation was impossible. Figure 1 shows such a flame anchored entirely to the nitrogen atom-containing wake of the plasma stream.

A new observation was the total disappearance of soot in the presence of the plasma, even when the latter was of argon alone and even in otherwise heavily sooting conditions. To achieve soot formation, toluene was injected with the propane fuel into the atomiser of the combustion chamber and Fig. 2a gives some impression of the luminosity of the flame which accompanied the heavy sooting. The result of injecting the argon plasma (Fig. 2b) is shown chiefly because colour photographs could not be published: the nitrogen-containing plasma (Fig. 2c) removes soot completely, probably under even more extreme conditions than does the argon plasma. There is, however, some luminosity visible in Fig. 2c: a greenish-yellow glow (also apparent under the conditions of Fig. 1) which is thought to result from some of the reactions of nitrogen atoms. This glow is seen to spiral down the length of the combustion chamber, because of the swirl vanes. Carbon luminosity disappears entirely in all cases, though black and white photographs do not show these distinctions.

Both chemical and physical mechanisms could account for soot suppression and the results, at this stage, do not allow us to attribute the effect uniquely to one or the other. Oxygen atoms (see below) and the OH produced in the next part of the radical chain could have this effect<sup>7</sup> but free electrons, which would be abundant in the plasma, have also been shown active in this way<sup>8</sup>.

It has been suggested that as regards flame stabilisation, at least, one of the relevant reaction steps is



There are likely to be several others, and even radiation from the plasma may exercise an effect. For example, it has been shown<sup>6</sup> that argon, though much less effective than nitrogen, is far from acting as an inert additive. Because of its exceedingly high temperature, a variety of reactions can occur at its interface with the other reactants; acetylene, for example, was found<sup>6</sup> when an argon plasma was injected into methane/air mixtures. In view of the effectiveness of nitrogen, however, it became desirable to consider whether the injection of nitrogen-containing plasmas would aggravate NO formation. Plasma-generated

In view of the success of these laboratory experiments, a car-battery operated plasma jet was tested in the exhaust system of a 1,200 cc saloon car and a considerable reduction of NO emission was achieved.

How flame stabilisation and NO removal can be associated becomes a little clearer if we consider the temperature dependence of the above-mentioned reactions. If one considers reaction (1) to be the main competitor for N atoms, and neglects the reverse of (1) and (2), as well as routes involving N atoms leading indirectly to nitric oxide, the following expression results

$$-d[\text{NO}]/dt = [\text{N}]\{k_2[\text{NO}] - k_1[\text{O}_2]\}$$

Therefore if  $k_2[\text{NO}] > k_1[\text{O}_2]$ , then nitric oxide may be destroyed at a rate proportional to  $[\text{N}]$ .

The temperature dependence of  $k_1$  ( $6.43 \times 10^9 T \exp(-6250/RT)$ ) and  $k_2$  ( $3 \times 10^{13} \exp(-334/RT)$ ) are shown in Fig. 3a; the low activation energy of reaction (2) makes it insensitive to temperature in the range of interest. When  $k_2[\text{NO}] = k_1[\text{O}_2]$  there will be no nitric oxide reduction. By plotting  $k_1[\text{O}_2]/k_2$  against temperature (Fig. 3b) it is possible to estimate a value of the lowest concentration of nitric oxide that could be established by N atom injection into nitric oxide-containing products (where the concentration was greater than the local steady state value of NO at a given temperature).

It is thus possible to postulate practical systems in which one plasma jet could fulfil both functions, one in the hot reaction zone and the other in the colder exhaust stream. This might be achieved, for example, by maintaining the discharge during parts of the combustion and exhaust cycle in a reciprocating engine, and by a suitable vortex in a continuous flow chamber.

Our current work is concerned with the analysis, by spectrographic and by other means, of the reactions occurring at the interface between plasmas of various constitutions and gases relevant in the combustion process. It seems already from these preliminary practical results, however, that it may be possible to design combustion systems in such a way that a small addition of electrical energy will facilitate the faster combustion of leaner mixtures simultaneously with the prevention of soot and nitric oxide in the products.

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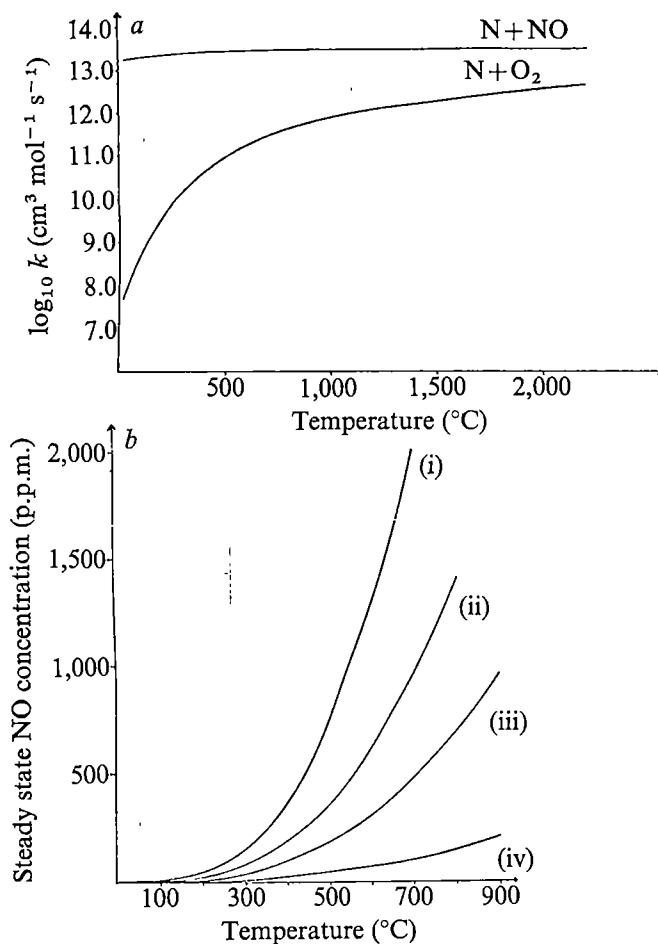
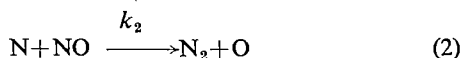


Fig. 3 Comparison of rates of reaction 1 and 2. a, Temperature dependences of rates constants; b, minimum nitric oxide concentration attainable for O<sub>2</sub> (i) 21% air (leanest limit), for example, an unthrottled engine at low load; (ii) 10%, unthrottled engine at high load; (iii) 5%, throttled engine, low load, high air/fuel ratio; (iv) 1%, throttled engine, idle or high load.

N atoms should, however, be able to reduce nitric oxide emissions by reversing the rate-determining Zel'dovich<sup>9</sup> step



To demonstrate this reaction taking place at atmospheric pressure, N atoms from the plasma were injected into fast flowing streams of nitric oxide in argon and synthetic exhaust gas mixes. Reductions in nitric oxide from 3,000 p.p.m. to a residual 80 p.p.m. were readily obtained in flows up to 250 l min<sup>-1</sup>.

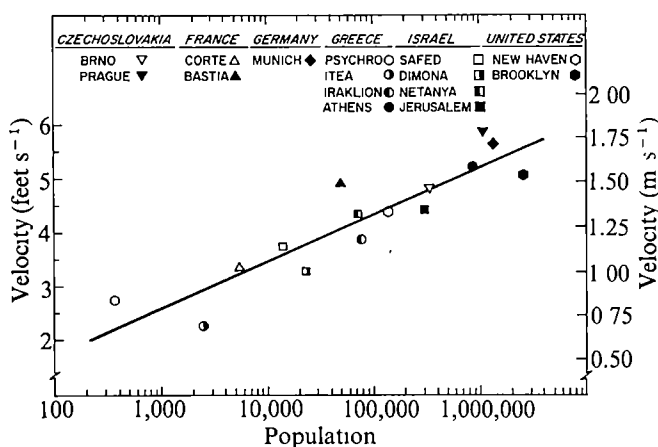
## The pace of life

THE specific effects of population pressure on the quality of everyday life should be of pressing social and policy concern; and although population studies have proliferated in the behavioural sciences, research has focused primarily on fertility-related behaviours<sup>1,2</sup>. Moreover, the few social scientists interested in the relationship of the numbers of humans to individual human behaviour have been puzzled by a dearth of clear-cut effects<sup>3</sup>. This study reports preliminary analyses from a larger cross-cultural investigation of the quality of life. Following a suggestion from Lowin *et al.*<sup>4</sup>, we have systematically observed the rates of pedestrian locomotion over a constant distance in

15 cities and towns in six countries in Europe, Asia and North America. The results of these observations indicate that pace of life varies in a regular fashion with the size of the local population, regardless of the cultural setting.

Between 1972 and 1974 we had the opportunity to visit and collect observational data on the quality and pace of life from several varied locations on three continents. As part of these studies we sampled and measured the tempo at which local inhabitants simply walked the main streets of their cities and towns. Walking represents a standard human activity which previous research had suggested might be subject to the influence of local population size<sup>4</sup>. The locations in which data were collected were chosen to represent functionally parallel sites, 'downtown' or commercial areas like Wenceslas Square in Prague, Flatbush Avenue in Brooklyn, and Rehov Yerushalaim in Safed. In each location 50 feet (15.24 m) along the main walkway were first measured and then passers-by were timed unobtrusively from a vantage point opposite the walkway. All the data were collected on dry, sunny days of moderate temperatures (mean 24.1 °C). Subjects ( $N=309$ ) were selected from those who walked the distance alone and unencumbered. They were selected unsystematically within these constraints and, in all cases but one, equal numbers were chosen from people walking in each direction. The data for each setting were preserved in raw form until observations on all of the 15 sites had been collected.

Figure 1 shows the mean velocities at which the inhabitants of the cities and towns traversed a constant 50-foot



**Fig. 1** Walking velocity (over 50 feet) as a function of population size. The line drawn through the data is the calculated least squares fit (linear-log regression): coefficient of determination ( $r^2$ ) = 0.82 Slope,  $V = 0.86 \log P + 0.05$  ( $V$ , velocity;  $P$ , population). Population values (pop.) were derived from statistical abstracts or government guides;  $n$  is the number in a sample;  $t$  is the mean time (s) ( $\pm 1$  s.d.) to walk 50 feet: Czechoslovakia: Brno (pop., 341,948,  $n = 20$ ,  $t = 10.4 \pm 1.8$ ) and Prague (pop., 1,092,759,  $n = 20$ ,  $t = 8.5 \pm 1.3$ ) *Statistická ročenka CSSR* (SNTL, Prague, 1974); France: Corte (pop., 5,491,  $n = 13$ ,  $t = 15.1 \pm 1.8$ ) *Guide Michelin* (Pneu Michelin, Paris, 1973) and Bastia (pop., 49,375,  $n = 26$ ,  $t = 10.2 \pm 1.1$ ) *Annuaire Statistique de la France* (Institut National de la Statistique et des Études Économiques, Paris, 1973); Germany: Munich (pop., 1,340,000,  $n = 30$ ,  $t = 8.9 \pm 1.3$ ) *Statistisches Jahrbuch für die Bundesrepublik Deutschland* (W. Kohlhammer, Stuttgart, 1973); Greece: Psychro (pop., = 365,  $n = 10$ ,  $t = 18.1 \pm 4.1$ ) Crete by J. M. Christoforakis (S. Alexiou Sons, Heraklion, nd) and Itea (pop., 2,500,  $n = 10$ ,  $t = 22.0 \pm 8.6$ ), Iraklion (pop., 78,200,  $n = 20$ ,  $t = 13.0 \pm 2.5$ ), and Athens (pop., 867,023,  $n = 20$ ,  $t = 9.6 \pm 1.4$ ) *Statistical Yearbook of Greece* (Government Printer, Athens, 1973); Israel: Safed (pop., 14,000,  $n = 20$ ,  $t = 13.5 \pm 2.5$ ), Dimona (pop., 23,700,  $n = 20$ ,  $t = 15.3 \pm 10.6$ ), Netanya (pop., 70,700,  $n = 20$ ,  $t = 11.6 \pm 2.4$ ), and Jerusalem (pop., 304,500,  $n = 30$ ,  $t = 11.3 \pm 1.4$ ) *Statistical Abstract of Israel* (Government Printer, Jerusalem, 1973); and United States: New Haven, Connecticut (pop., 138,000,  $n = 20$ ,  $t = 11.4 \pm 1.8$ ), and Brooklyn, New York (pop., 2,602,000,  $n = 30$ ,  $t = 9.9 \pm 1.0$ ) *Statistical Abstract of the United States* (US Government Printing Office, Washington, 1973).

distance. Statistical analyses showed significant variation among sample mean times to walk the distance ( $F=14.69$ , d.f.=14/84,  $P < 0.001$ ). In Fig. 1, velocity is plotted against log population for the location (the total number of people living within the city limits<sup>5,6</sup>). As can be seen, the relationship between walking velocity and size of the local population is suitably characterised as linear, and the coefficient of correlation between these variables ( $r=0.91$ ) is significant beyond the 0.001 level of confidence (two-tailed test). These results suggest that a highly predictable relationship exists between the pace of life which characterises a locale and the size of its population.

Further, a comparison of cities with towns (breakpoint for a Standard Metropolitan Statistical Area<sup>7</sup> > 200,000) shows that average walking time for city dwellers (mean = 9.8 s) significantly exceeded that of their town counterparts (mean = 13.4 s) ( $t=7.66$ , d.f.=307,  $P < 0.001$ ). Similarly, nearly every intranational comparison indicated that city dwellers move at significantly greater speeds than their smaller town compatriots. Both these analyses further corroborate the notion that city life, at least in these Western locations, is carried on at an increased tempo<sup>4</sup>.

What explains the regularity with which locomotion velocity and population covary? Recent analyses of population pressures on behaviour suggest that "the number of individuals who must interact, rather than density, is the variable that produces substantial effects on human behaviour."<sup>8</sup> In addition, the crowding that accompanies larger groups of humans has been defined operationally to include not only numbers of people, but also the perception of personal restriction<sup>8</sup> and increased levels of social stimulation<sup>9</sup>. Both latter concepts covary with the size of the population. In view of this, crowding has been thought to motivate specific timing and avoidance behaviours that reduce tension and social interference. Milgram<sup>10</sup>, for example, has proposed an organising theory of psychological behaviour in cities that is based on a systems concept directly related to this view of crowding. According to his "overload" hypothesis, the volume of stimulation which characterises highly populated areas and typically overloads the processing capacity of the individual is reduced by cognitive and behavioural adaptations—usually withdrawal responses—which in turn facilitate purposeful action. These adaptive mechanisms eventually evolve into behavioural norms and, assuming this interpretation, increased walking speeds serve to minimise environmental stimulation. The data on the pace of life presented here reflect an adaptation to the continuum of population effects<sup>10,11</sup>. Thus, although our samples are relatively small and the communities were not randomly selected, our data suggest that the immediate social and physical environment exert a strong control over individual behaviour. They imply that demographic circumstances affect the quality of life, psychological experience, and motor action of humans in a fairly predictable manner, even in varied cultures.

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## Mortality and the 1974 fuel crisis

SOME comment is needed on the report by Brown *et al.*<sup>1</sup> in which the authors claim that the low mortality they noted in San Francisco and Alameda Counties in the first quarter of 1974 might have arisen from the reduction of gasoline sales during the fuel crisis. This is an interesting proposition, but the further details that the authors are examining on the more usual determinants of seasonal and secular variations in mortality (weather conditions and the prevalence of respiratory infections), and on urban/rural comparisons will be required before any judgment can be made on possible cause-and-effect relationships. In the meantime we make a few comments on their work, and present some related data from Britain.

Brown *et al.* have provided very little information on which to assess the significance of the variations in quarterly death rates. The actual numbers of deaths involved have not been quoted (although these can be estimated from a knowledge of the total populations), and beyond stating that the first-quarter rates for the years 1970–73 were “rather homogeneous”, there is little indication of the extent of year-to-year fluctuations.

The comment that the greatest change occurred in the under-45 age group, and the least among those aged over 65 is surprising, in view of the low death rates in the former group. Normally, mortality in the over-65 group dominates the total death rates, and it is this group that shows the greatest changes in mortality in epidemics of influenza or in extreme weather conditions. Presumably age-specific death rates had been calculated, but these were not shown. Although it is unlikely that there would have been any change in age distribution within either area during the five years concerned sufficient to affect comparisons of total death rates from one year to another, the contrast in death rates between San Francisco and Alameda Counties suggests a major difference in age distribution between these two areas.

In view of their suggestion that the effect on mortality is related to the concentrations of pollutants from motor vehicles, it is surprising that results of measurements of, for example, carbon monoxide, have not been quoted. Apart from variations in the emission of pollutants from one period to another, the prevailing weather conditions are important in determining the actual concentrations found in streets.

**Table 1** Annual death rates in Greater London for 1971–75

Year	Annual death rates* per thousand for the quarters ending			
	March	June	Sept.	Dec.
1971	12.8	11.2	10.0	11.7
1972	13.5	11.1	10.1	12.4
1973	13.3	11.0	9.9	12.0
1974	13.3	11.1	10.3	12.0
1975	13	12	10	—

\*Death rates as published by the Office of Population Censuses and Surveys<sup>2</sup>, subject to modification in relation to revised estimates of population, and to final adjustments of deaths in relation to exact date and place of occurrence. The numbers of deaths per quarter range from 18,000 to 25,000.

**Table 2** Annual death rates in Greater London for 1955–57

Year	Annual death rates* per thousand for the quarters ending			
	March	June	Sept.	Dec.
1955	14.3	9.7	8.2	10.4
1956	14.8	9.7	8.4	10.6
1957	11.3	9.7	8.7	13.2

\*Death rates as published in the Registrar General's Quarterly Returns<sup>2</sup>. The numbers of deaths per quarter range from 17,000 to 30,000. Greater London was not identified separately in the records in other years around this period, and the definition of the area differs slightly from that in Table 1.

Earlier work by Hexter and Goldsmith<sup>3</sup>, based on daily mortality records in Los Angeles County, showed that a model including regression on the logarithm of the daily mean concentration of carbon monoxide was capable of accounting for a marginally greater proportion of the variance in deaths than one involving trend, cyclic variations and temperature, without added pollution variables. The interpretation of such findings is, however, difficult since some of the variables involved are correlated with one another, and in these circumstances ordinary least squares estimates of the regression coefficients are unsatisfactory<sup>3</sup>.

Relationships between daily mortality and concentrations of pollutants from heating sources (smoke and sulphur dioxide) have been studied in London<sup>4–6</sup>, but the possibility of associations with pollutants from traffic have not so far been considered. Inspection of the records of death rates for each quarter of the year as published for Greater London<sup>7</sup> for the past five years (Table 1) does not reveal any reduction in mortality in the first quarter of 1974 when, as in California, there was a marked reduction in the use of cars as a result of the fuel crisis (and a more important feature in London, affecting the emission of pollution both from heating and from vehicles was the limitation in the use of fuels for heating and power, with the imposition of a three-day working week).

It is of interest also to consider an earlier period, during the Suez crisis in the early months of 1957, when as a result of petrol rationing there was a major reduction in the use of cars, and substantial changes in carbon monoxide concentrations were noted in central London<sup>8</sup>. The death rates for Greater London on a quarterly basis during the three years 1955–57 (ref. 9) are shown in Table 2.

The relatively low mortality rate in the first quarter of 1957, and the high mortality rate in the last quarter were features common to the records for the whole of England and Wales. The rise at the end of the year was related to the Asian influenza epidemic, and the low death rate in the first quarter was attributed to the mild weather<sup>10</sup>. There have been other March quarters in recent decades with low mortality (for example 1948, 1935) and some with high mortality, related to cold weather (as in 1963 (see ref. 11)) or to influenza epidemics. Further evidence of the important effects of temperature on deaths from heart disease has been reported by Bull and Morton<sup>12</sup>. Much caution is therefore required before ascribing changes in mortality to other environmental factors, and it is necessary to examine a long series of data with influenza epidemics, periods of extreme weather conditions, or episodes of unusually high or low pollution in a variety of combinations with one another before the relative importance of each of these factors can be judged satisfactorily.

In addition to the published quarterly and weekly returns of deaths, special daily tabulations have been prepared for London for a number of years, and more recently these have been extended to other urban areas and to England



and Wales as a whole. A detailed analysis of these is already in progress, and some of the findings on the effects of several environmental factors will be published shortly. Meanwhile we await with great interest the further results and analyses promised by the Berkeley workers.

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THE paper by Waller and Lawther<sup>1</sup> commenting on our report of a decrease in mortality during the 1974 fuel crisis<sup>2</sup> raises some interesting and important points. We chose two separate counties to examine the possible effects on mortality of the fuel crisis for the purposes of testing consistency. Although both Alameda and San Francisco Counties are in the San Francisco Bay Area, they vary somewhat in age and racial composition, in density of population, in occupational structure, and in weather conditions. Yet both counties showed a drop in mortality during the first quarter of 1974.

Why no such mortality decrease was seen in London

on the basis of variation in such factors, but we have not yet investigated the possible interaction of all these factors.

A further possibility is that the decrease in mortality is part of a secular trend. We should be in a better position to answer this question when later data become available.

Waller and Lawther suggest that measurements of specific pollutants ought to have been presented. For several reasons we decided that our investigation should not hinge on variations in observed pollutant levels. First, we have become aware of significant technical problems in the actual measurement and recording of pollutant levels. Further, in San Francisco and Alameda Counties, there are only five air-sampling stations for a total population of one-and-three-quarter million people. Thus, reported pollutant levels are at best a very indirect guide to human exposure. Also, one must distinguish between the exposure of the general population to ambient levels of pollutants and the localised over-exposure of people in and around motor vehicles. We do, however, intend to publish ambient pollutant levels in the larger paper to follow. In response to Waller and Lawther's other questions: the number and rates of deaths per quarter are given in Table 1.

It should be stressed that the data from the two counties were collected differently. The Alameda County deaths are reported by place of occurrence, and for that county the figures in Table 1 should be accurate. The San Francisco deaths are reported by place of residence. As the San Francisco figures are from early reports, they may not include some deaths among San Francisco residents who died out of the county, and death reports received by the Registry after March 31, 1974. When these latter deaths are included, the 1974 mortality figure for San Francisco (but not for Alameda) may be higher than the one quoted.

Waller and Lawther are correct in noting the great absolute difference in the death rates between these two counties. This difference cannot be wholly explained by the older age distribution in San Francisco, but is presumably the result of other ethnic and environmental factors. We

**Table 1** First quarter deaths and death rates per 100,000 population. All causes excluding motor vehicle accidents

	1970	1971	1972	1973	1974
<b>Alameda County*</b>					
Number of deaths	2,122	2,184	2,102	2,256	2,019
Quarterly mortality rate	225.3	230.0	219.3	234.2	209.7
<b>San Francisco County</b>					
Number of deaths	2,682	2,610	2,631	2,608	2,221
Quarterly mortality rate	374.8	371.3	381.6	382.7	327.0

\* Excludes the cities of Berkeley and Albany, which have separate death registries.

during the first quarter of 1974 is not clear. One may speculate that the three-day working week and the consequent social disruption, the apparent decrease in home heating, and many other factors might have affected overall mortality in a way that would 'mask' any effect on mortality of the decrease in gasoline consumption.

The other possibility for the discrepancy between our findings and the London findings is that raised by Waller and Lawther, that is, factors other than the change in gasoline consumption may have been responsible for the decrease in mortality (in the San Francisco Bay Area). We have examined separately the variation in rainfall, temperature and air stability that occurred in the first quarters of 1970-74 and in each case 1974 was fairly typical. Similarly, the change in the number of deaths from influenza and pneumonia over the 1970-74 period does not seem to vary with the mortality rates of the counties in question. We have not been able to explain the decreased mortality in 1974

have taken account of the change from year to year in the population size of each county, but not of any change in internal composition, as such figures were not available to us. Our assertion that the greatest proportionate change occurred for those under the age of 45 is based on the percentage change using the average death rates for the comparison years as baseline. Of course, the absolute rates are much higher in those over the age of 65 but the greatest proportionate change was in the younger age groups. This preliminary analysis of the effect within age strata was not simultaneously tested by race and sex; consequently, no clear-cut conclusions can be drawn until this is accomplished.

We are heartened that other investigators have begun to look at populations and areas very different from our own to corroborate or refute our findings. We sincerely hope that these efforts will continue. We would hope that analysis by cause of death, and of the other factors which may mag-

nify or diminish observable changes in mortality during the fuel crisis period will also continue.

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## Wild mynahs mimic wild primates

THE remarkable accuracy with which captive hill mynahs (*Gracula religiosa*) mimic human speech and other sounds is well known<sup>1</sup>. How this ability functions in nature was entirely speculative until Bertram<sup>2</sup> conducted an extensive field study of hill mynahs in India. Bertram<sup>2</sup> found that hill mynahs in India imitate the calls of other hill mynahs, but never heard a wild mynah imitate another species.

A different situation prevails on Siberut Island, off the west coast of Sumatra, Indonesia. During a 14-week study (1 July–7 October, 1972) of primates in rain forest near the Sirimuri River in southern Siberut<sup>3</sup>, I noted several instances of wild *G. religiosa* imitating a particular loud alarm call of Kloss's gibbon (*Hylobates klossii*) and loud spacing call of male pig-tailed langurs (*Simias concolor*).

I saw hill mynahs daily in the study area, usually in groups of 2–10 individuals. The gibbon call that they imitated is a tonal trill at a frequency of ~1 kHz and an average duration of 1 s (21 sonagrams). The langur call that they mimic is a panting-like sound, produced by 3–5, or more, exhalation–inhalation cycles, at a frequency of ~500 Hz (1 sonagram). Mynah imitations of these calls sound much like the originals but are distinguishable by ear as being mynah calls rather than primate calls just as readily as mynah renditions of human speech can be recognised by ear.

Mynahs imitated calls immediately after the primates produced them. From 16–30 September, 1972, I noted the frequency with which male pig-tailed langur calls were followed by mynah imitations of the calls. Out of 12 instances of calling by one or more male pig-tailed langurs, 10 were followed within seconds by imitations of the call by one or more mynahs. Comparable data were not obtained for mimicking of gibbon alarm trills because I heard none during this period.

Neither gibbon alarm trills nor the male pig-tailed langur call was a common component of the forest background noise during this study. Only 12 bouts of gibbon alarm trills, distributed over 8 days, were heard during the 99 days of study. Pig-tailed langur calls were never heard during the first 11 weeks of the study, but occurred on two-thirds of the days during the final three weeks of study. R. L. Tilson (in preparation) has confirmed that calling by pig-tailed langurs occurs sporadically.

Three kinds of loud primate calls are, at particular times of day, regular components of background noise in the study area. These are the songs of male and female Kloss's gibbons and spacing calls of male Mentawai langurs (*Presbytis potenziani*). None of these calls was imitated by mynahs, in spite of the fact that the Mentawai langur call is rather similar to the pig-tailed langur call which is mimicked. This suggests that perhaps only infrequently occurring vocal signals of other species are mimicked by mynahs. I did not determine whether mynahs imitate vocalisations of other species of birds in the study area.

These findings suggest that such mimicking behaviour is not, as previously thought<sup>2</sup>, just an artefact of confinement

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## Wild birds detect quinine on artificial Batesian models

EVIDENCE I acquired during an experiment on the evolution of Batesian mimicry indicated that under some circumstances birds can detect quinine dihydrochloride on flour–lard pastry baits. The results of some previous mimicry experiments<sup>1–5</sup>, in which pastry baits were presented to wild birds, may need to be re-evaluated because of their tacit assumption that birds can only detect by taste the quinine used to make the models unpalatable. Quinine salts used were the hydrochloride<sup>2,3</sup> or dihydrochloride<sup>1,5</sup>. Pilecki and O'Donald's "solution of quinine"<sup>4</sup> was presumably quinine hydrochloride, which they used previously. Quinine monohydrochloride has only 1:16 solubility<sup>6</sup>, so Morrell and Turner<sup>2</sup> probably used the more soluble dihydrochloride to make their 75% solution. Birds in an experiment of Ikin and Turner<sup>1</sup> took nearly as many perfect mimics of two different models as palatable controls, though very few models (70% quinine) were taken. These authors suggested that birds in previous experiments<sup>2,5</sup> may have distinguished "models from 'perfect' mimics, possibly because the quinine causes a slight yellowing of the pastry". So far as I can determine, however, a specific control for detection of quinine has never been conducted.

The intent of my original experiment was to test the hypothesis that the models evolve more slowly than the mimics due to stabilising selection<sup>7</sup>; that is, deviant individual models are subject to predation because birds fail to recognise them as unpalatable. Until my suspicions about detection of quinine by birds were aroused, I used the following procedure in the study. Birds were trained to avoid unpalatable yellow models and palatable (Batesian) yellow mimics, and to choose palatable pink controls. All baits were painted with black patterns. The position of a single black stripe distinguished models from mimics, while controls were painted with four other patterns. I planned next to test avoidance of models with altered black patterns. During training, two scrub jays (*Aphelocoma coerulescens*) avoided yellow model baits treated with a 5% quinine solution (Q-yellow) and yellow mimics (non-Q-yellow, dipped in distilled water). They chose controls (non-Q-pink, distilled water) significantly more than models and mimics combined ( $\chi^2 = 127.12$ ,  $P < 0.001$ , see Table 1b). Other birds feeding on the baits—6–20 Golden-crowned sparrows (*Zonotrichia atricapilla*), two to six brown towhees (*Pipilo fuscus*), one to two rufous-sided towhees (*P. erythrophthalmus*) and one to two California thrashers (*Toxostoma redivivum*)—did not discriminate among model, mimic and control. I increased the unpalatability of the model, using 60% quinine dihydrochloride for the rest of the experiment. The birds other than jays then chose non-Q-yellow mimics significantly more than Q-yellow models ( $\chi^2 = 29.35$ ,  $P < 0.001$ , Table 1c). The jays continued to avoid both Q and non-Q-yellow baits significantly.

The first indication that some birds detected quinine visually or olfactorily was that only 12 Q-yellow baits were taken during the 4 d when 60% quinine was used initially, although at least 17 birds in addition to jays were seen feeding on the first day alone (Table 1c). I was certain that the birds did not taste baits without attacking them. I used field glasses to watch

Table 1 Experimental details and results

Date (1973)	Model	Mimic	Control	No. presented	No. taken, all birds	No. taken, scrub jays only
(a) January 19, 20, 22-24	5% Q-yellow			250	109	39
			non-Q-pink	250	202	127
(b) January 27, 28, 31	5% Q-yellow			300	66	
		non-Q-yellow		300	76	2 yellow*
			non-Q-pink	300	218	133
(c) February 15-18	Q-yellow			200	12	0
		non-Q-yellow		200	57	0
			non-Q-pink	200	160	102
(d) March 15	Q-pink			50	0	0
		non-Q-pink		50	43	33 pink*
			non-Q-green†	50	21	16

Tests were run at Stanford between 0800 and 1000 each fair day between January 19 and March 28, 1973, and sessions were continued for 20 min or until about 10 of the most heavily predated class of baits remained. Baits were presented in random order, 10 cm apart in a rectangular or square array on a secluded lawn, and were not replaced when taken. Feeding was continuously observed during sessions; the number of each colour taken by each species was recorded. A test for colour preference had been conducted for 5 d before January 19. Given 50 unpatterned non-Q-pink and 50 unpatterned non-Q-yellow baits per session, the birds ate more of the latter, although the difference was not significant. Models were then designated yellow. Treatment of models was with 60% quinine, except during (a) and (b).

\*Q and non-Q indistinguishable through field glasses, but jays must have taken only non-Q-pink during (d), since no models were taken by any birds.

†Green baits were not cryptic on the dormant grass.

Table 2 Quinine detection test

Date (1973)	Unpatterned baits	No. presented	No. taken, all birds	No. taken, scrub jays only
March 22	Q-pink	50	1	
	non-Q-pink	50	39	22*
March 28	Q-yellow	50	3	2†
	non-Q-yellow	50	20	0

Since mimics became the preferred prey in the previous test, non-Q controls made of different coloured pastry were no longer necessary. Differences shown are all significant at the 0.001 level except predation on yellow by jays.  $\chi^2$  values for predation on Q-pink against non-Q-pink: jays, 18.18; other birds, 14.22. For predation on Q-yellow against non-Q-yellow, by birds other than jays,  $\chi^2 = 17.19$ .

\*Indistinguishable, but at most one was Q-pink.

†Determined by my retrieving and tasting baits after jays dropped them

all feeding, and the birds never brought the tips of their beaks close to one bait and subsequently chose another, nor were there beak marks on baits that were not taken.

When it was obvious that a specific test for perception of quinine was necessary, the birds were trained for 3 d on unpatterned, non-Q-pink and non-Q-green baits to accustom them to new green controls. I then tested avoidance of a new model—Q-pink baits painted with the same black pattern as the previous yellow model. All birds avoided them (Table 1d). Although they had never before seen a quinine-treated pink bait, these birds could distinguish between Q-pink and non-Q-baits. To determine whether this discrimination was made by learning the patterns that had been painted on the baits or by perceiving the quinine, unpatterned pink baits—50 Q and 50 non-Q—were presented in random order, 10 cm apart in a square array. Position within the square identified uneaten baits as to treatment; baits not taken remained undisturbed by the birds. Table 2 shows that discrimination was based solely on the presence of quinine. The test was repeated using unpatterned Q-yellow and non-Q-yellow baits. Although the jays had so far taken a mean of 36.5 baits per session, they took only two baits, subsequently identified as quinine-treated. The other birds avoided the Q-yellow baits ( $P < 0.001$ ).

Clearly these birds detected 60% quinine-treated baits without tasting. Since jays distinguished Q from non-Q baits when the pastry was pink and were apparently unable to do so with yellow pastry, their discrimination was probably made on a visual, rather than an olfactory basis. That jays took only non-Q-pink controls during the aborted experiment (Table 1c) was a further indication that they could not distinguish between Q-yellow and non-Q-yellow baits, although the other

birds could do so with 60% quinine. Future experiments on mimicry using pastry baits must include a control to determine that the predators cannot perceive the unpalatable substance itself if baits are merely dipped into a solution. The unpalatable compound may have to be injected into baits, as Alcock<sup>8</sup> did with quinine-treated mealworms, or otherwise hidden within the pastry. This is effective if the birds do not swallow food whole, but break it up and thus taste the quinine. For birds to learn to avoid a model, it must have a punishing aspect, either an unpleasant taste or a distinct taste combined with emesis, such as quinine sulphate<sup>9</sup> provides.

The birds in my investigation made a very fine discrimination; I could not see the quinine on the baits. Certainly birds can and do make choices based on very small differences between food items; for example, cliff swallows have been known to select the non-stinging drones among flying bees<sup>10</sup>. It has, however, been pointed out<sup>11</sup> that predators do not need to avoid models and mimics completely for mimicry to evolve—only that better mimics must be avoided more frequently than less perfect mimics.

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## Aggregation response of nymphs to pheromone(s) produced by males of the tick *Amblyomma hebraeum* (Koch)

No aggregation response by tick nymphs to a pheromone(s) produced by males had been described, although such a response had been demonstrated in adults<sup>1-3</sup>. We have now shown that males of the hard tick *Amblyomma hebraeum* (Koch) produce a material(s) which induces aggregation of nymphs suggesting an assembly pheromone(s) (Fig. 1). The presence of this pheromone is demonstrated by biological observations and by experimentation with a choice chamber<sup>4</sup>.

Males of *A. hebraeum* which readily attach to the host, were placed on the ears and on shaved areas of the back of rabbits (*Lepus europaeus* Pallas) and on the ears and scrotum of Guernsey calves. Hexane extracts were made by washing males of *A. hebraeum*, which had previously fed for 8 d on a calf. The extracts were concentrated to 1 ml (100 males) by vacuum distillation and were applied, drop by drop, discrete areas (a circle about 3 cm in diameter) on the ears and backs of rabbits, and on the ears

Fig. 1 Assembly of *Amblyomma hebraeum* nymphs (a) to male ticks which had attached to the host 8 d previously (b), to hexane extract of fed males. The method of extraction was described previously<sup>5</sup>.

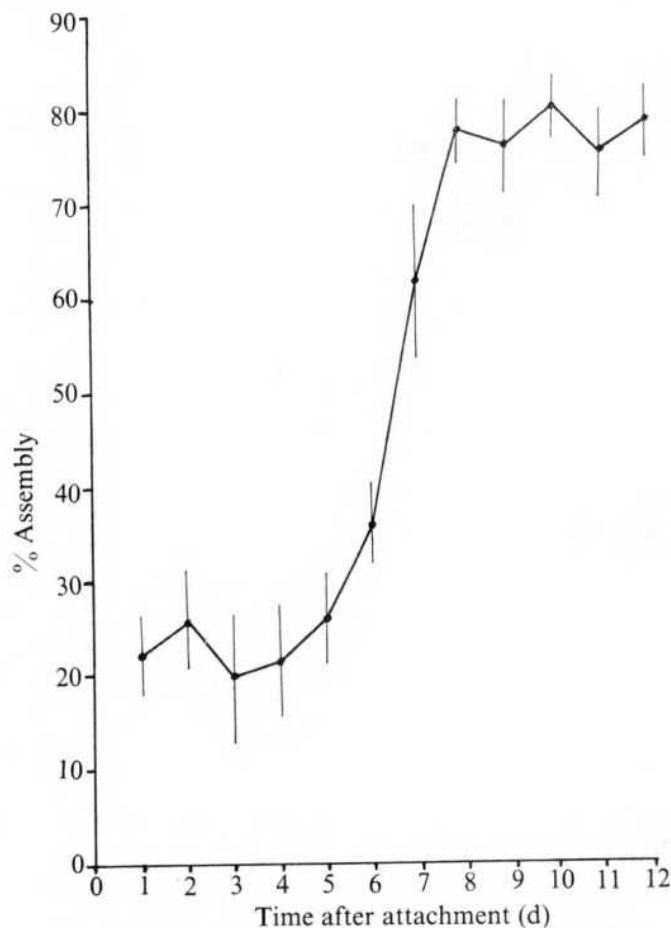
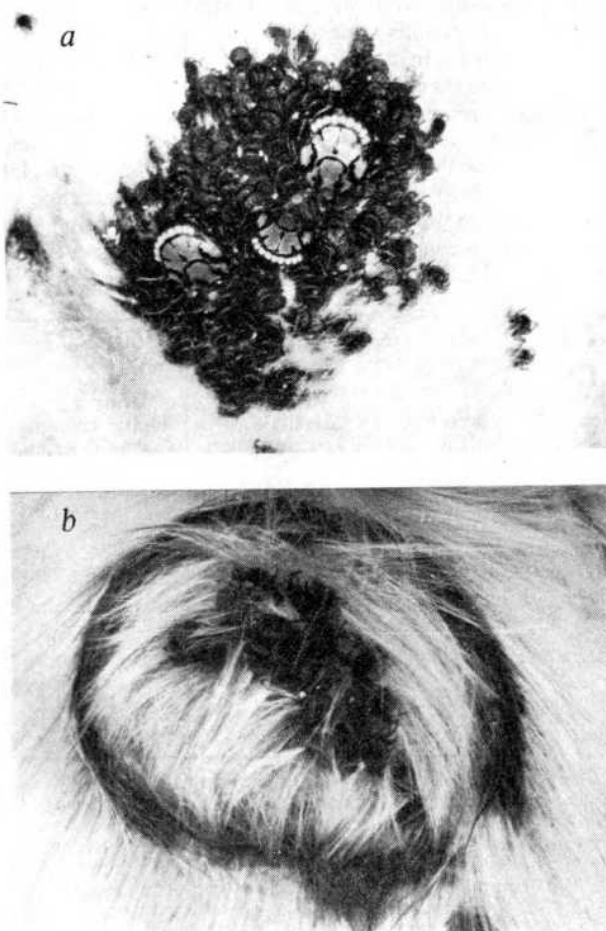


Fig. 2 Percentage assembly of *A. hebraeum* nymphs to males after various periods of feeding on scrotum of calves. Vertical line represents s.e.m.

and scrotum of calves. Test areas were enclosed with large cloth sleeves and a relatively large number of unfed nymphs was deposited in the sleeves. After 24 h the sleeves were removed and the number and area of attachment of the nymphs were recorded (Table 1). No nymphs released on a host aggregated around females which had attached to a host 5 d previously or to nymphs which had attached 4 d previously.

As a control, nymphs were exposed in a similar manner to hosts which were not infested with males or were not treated with hexane extracts of males. On control hosts, nymphs attached readily, but were evenly distributed through the area covered by the cloth sleeves and no clusters of attaching nymphs were formed.

As free blood or damaged tissues around the site of attachment might have resulted in attraction of nymphs, wounds were created on the ears of rabbits using a sharp needle. Nymphs in cloth sleeves exposed to ears treated in this way showed no clustering of attaching nymphs to either free blood or damaged areas.

The time required by nymphs for attachment to the hosts on which males were attached, or to areas of the hosts which had been treated with hexane extracts of fed males, was significantly ( $P < 0.005$ ) shorter than in untreated control animals. The percentage of males emerging from nymphs which developed on hosts which had males attached, was compared with the percentage of males emerging from nymphs which developed on control animals. From all the tests examined  $46.4 \pm 8.21\%$  were males from nymphs which attached in clusters in association with a male and  $49.4 \pm 6.85\%$  were males from those that emerged from nymphs which had not aggregated around a male on the host. This small difference was subjected to statistical analysis using the  $t$  test and found to be not significant.



Table 1 Rate of assembly of *A. hebraeum* nymphs around *A. hebraeum* males

Area of host	% Assembly of nymphs (mean $\pm$ s.e.) around males which had attached to the hosts 8 d previously	% Assembly of nymphs (mean $\pm$ s.e.) attached to area where hexane extract of males was previously applied	% Assembly of nymphs (mean $\pm$ s.e.) attached to area where pure hexane was previously applied
Rabbit ear	82.1 $\pm$ 4.02	86.5 $\pm$ 3.72	7.2 $\pm$ 1.78
Rabbit back	93.3 $\pm$ 1.71	92.1 $\pm$ 2.01	9.2 $\pm$ 3.12
Calf ear	93.6 $\pm$ 1.60	84.4 $\pm$ 3.86	6.5 $\pm$ 2.08
Calf scrotum	79.5 $\pm$ 3.34	90.9 $\pm$ 4.72	2.9 $\pm$ 1.45

This clearly indicates that all nymphs are attracted to a mature male on the host, irrespective of whether they are destined to develop as males or females.

The unfed males on the host did not attract nymphs, so further examination was necessary to determine the duration of the feeding period before males would attract nymphs (Fig. 2). Larvae did not respond to the hexane extracts of adult males to which nymphs clearly responded.

In each of the tests in the choice-chamber 100 unfed nymphs were placed in the lower arm of a Y-shaped glass tube, the ends of which were enclosed with fine nylon mesh. By connecting a vacuum pump to the lower arm of the Y-tube an airflow was produced. Tick nymphs were introduced to the lower arm while test materials were placed on the anterior arms of the choice chamber. In each test two materials were compared and three counts of the distribution of the nymphs were made at 10-min intervals. After three counts all nymphs in the system were collected and reintroduced to the lower arm of the Y-tube. Each test was replicated six times. The various materials that were checked in the choice chamber were: fed males, partly engorged females, partly engorged nymphs, unfed males, unfed females, unfed nymphs, hexane extract of males and pure hexane.

The results of a series of tests showed significant ( $P < 0.002$ ;  $t$  test for paired observations) attraction of nymphs only in the tests in which fed males or hexane extract of fed males were present.

We have shown that males which fed for 8 d, or the hexane extract from such males significantly attracted nymphs, whereas males which had not been fed, or partially engorged females as well as partly engorged nymphs presented no attraction to nymphs.

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## Position-dependent development of tobacco meristems

THE apical meristems of angiosperms exhibit indeterminate growth during vegetative development and determinate growth in the production of thorns and flowers<sup>1</sup>. The mechanism(s) controlling the conversion from indeterminate

to determinate growth is poorly understood, although photoperiod and temperature have been shown in many plants to be important environmental signals for flower induction<sup>2</sup>. In 'day-neutral' plants, which are not strictly regulated by photoperiod, the conversion of their meristems to the determinate flowering condition seems to be endogenously regulated. We have examined this conversion in *Nicotiana tabacum* cv. Wisconsin 38, a day-neutral plant. Our data show that a uniform number of nodes is produced before conversion, and this pattern of meristem development is regulated by information supplied to the meristem by the plant.

Wisconsin 38 tobacco exhibits limited growth because its terminal meristem differentiates into a flower. It has strong apical dominance and its vegetative axillary buds are all arrested after the initiation of eight or nine leaf primordia. In certain environmental conditions the number of nodes produced by the terminal meristem is very uniform<sup>3</sup>, and we have observed this pattern of growth in numerous plants at various times of the year. In one set of thirty plants, an average of 35.4 nodes was produced (s.d. = 1.0).

We examined the conversion from indeterminate to determinate growth by taking the number of nodes produced by axillary buds (a single bud in the axil of each leaf) as a function of their position on the main axis. In our first experiments, plants at anthesis (first flower open) were decapitated at various internodes and the uppermost axillary bud was allowed to develop. These buds replaced approximately the number of nodes removed, with the higher buds producing fewer nodes than the lower buds (Table 1). This differential growth response might be related to the developmental state of the plant, and so we decapitated some plants at 3 stages before anthesis; when the inflorescence was visible, when the terminal meristem had just become reproductive, and when it was still vegetative (Table 1). In all cases the higher buds produced fewer nodes, suggesting that

Table 1 Nodes produced by terminal and axillary buds

State of development of decapitated plant*	Node number of bud that developed after decapitation	No. of nodes produced by bud in column two ( $\pm$ s.d.)
Anthesis (not decapitated)	Terminal	35.4 $\pm$ 1.0
Terminal meristem vegetative	1	42.8 $\pm$ 1.0
	9	28.3 $\pm$ 2.9
Reproductive terminal meristem just initiated	1	40.5 $\pm$ 1.7
	12	28.5 $\pm$ 0.6
Inflorescence visible	5	36.6 $\pm$ 1.9
	17	25.2 $\pm$ 2.5
Anthesis (first flower open)	5	39.0 $\pm$ 3.2
	17	23.2 $\pm$ 3.1
	22	13.8 $\pm$ 0.5

\*Plants were grown from seed under standard greenhouse conditions. Decapitations were made at the internode above the bud indicated and several of the axillary buds below this bud were removed so as to promote its growth. The first node is considered to be the node which produces the first leaf that hangs over the edge of a 7-inch pot. About five or six leaves are produced before this 'first' node. Nodes were numbered acropetally with node number 1 at the base.

developmental response of a bud is a function of its position on the stem. This position-dependent response could be a function of the age of the bud, of its actual position, or of the state of differentiation of the vascular connections between the bud and the main axis.

The developmental potential of isolated buds was examined by rooting buds without the use of exogenous phytohormones, and growing them to maturity. Twenty-five buds from various nodes of several plants produced 36.1 nodes (s.d.=3), indicating that they had no 'memory' of their original position, which suggests that a bud's pattern of development may depend on information received from the plant. To examine this, we grafted buds in four patterns; apical bud to apical internode, apical bud to basal internode, basal bud to basal internode, and basal bud to apical internode (Table 2). When the 21st bud was grafted to the 22nd internode and released from apical dominance by decapitation, it produced twice as many nodes as the intact 22nd bud released from apical dominance (25.3 as against 13.8), and when grafted to the 6th internode, about three times as many (37.3). A 5th bud grafted to a 6th internode produced about the same number as an intact 5th bud (41.5 as against 39.0) but  $\sim 1/3$  fewer when grafted to the 22nd internode (27.3). These results clearly show that the number of nodes produced can be altered by moving the bud up or down on the stem. The increased number of nodes produced by an apical bud that was grafted into an apical position was, however, puzzling. Since grafting involves significant injury as well as the establishment of new vascular connections, we examined the growth pattern of apical buds after severance of the vascular connections and tissues below the bud by a horizontal incision. As with grafting, this treatment resulted in an increased number of nodes (Table 2). The increased number of nodes produced is probably not primarily a response to injury, but rather reflects an altered movement of materials from the main axis to the bud and vice versa.

Our experiments have shown that the number of nodes produced by an intact axillary meristem is a function of its position on the stem. Three lines of evidence suggest that the apical meristem does not autonomously control the number of nodes produced; first, all rooted buds produce about the same number of nodes; second, grafted buds can produce more or fewer nodes than expected from the bud if it had remained intact; and third, when the tissues below a bud are cut, the bud produces more nodes than expected. Thus, the meristem seems to respond to information from the plant since the developmental pattern of a grafted bud can be altered by moving it up or down the plant. The increased number of nodes produced by grafting or cutting the tissues below an apical bud suggests, however, that appropriate transmission of this information may not occur through newly regenerated tissues. It is also possible that the temporary separation of the bud from the lower part of the plant may disrupt a positional information signal and thereby lead to an altered developmental response. In any event, the state of differentiation of the tissues connecting the bud with the main axis seems to be very important in maintaining developmental growth potential.

The observations that *N. tabacum* plants produce a uniform number of nodes in certain environmental conditions<sup>3</sup> and that various axillary buds from a single plant can express different growth potentials<sup>4,5</sup> are not unique. The experimental demonstration that the number of nodes produced by an organised meristem before commencing determinate growth is a function of the total number of nodes in the plant has not, however, been reported previously. This suggests that the terminal meristem (an axillary meristem—grafted or intact—becomes the terminal meristem after decapitation) receives information from the plant which orients the cells of the meristem in terms of their place on the main axis. This orientation in turn

Table 2 Nodes produced after grafting or wounding

Treatment	Node number of bud that developed†	No. of nodes produced by bud in column two ( $\pm$ s.d.)
Grafting*		
21st bud to 22nd internode	21	25.3 $\pm$ 2.1
21st bud to 6th internode	21	37.3 $\pm$ 1.3
5th bud to 6th internode	5	41.5 $\pm$ 2.6
5th bud to 22nd internode	5	27.7 $\pm$ 0.4
Wounding		
Decapitated—not wounded	26	15.8 $\pm$ 3.8
Decapitated—wounded below node 26†	26	22.5 $\pm$ 0.6

\*The bud to be grafted was removed by cutting round the bud through the vascular tissues to the pith and then lifting off the patch containing the bud. At the graft site the bud was slipped into a T-shaped cut and wrapped with parafilm for several days. After 10 d the plant was decapitated above the grafted bud.

†Plants were wounded by making a cut 1.5 cm below the bud and half way through the stem. Post-wound treatment was the same as for grafted buds.

‡Nodes were numbered acropetally with node number 1 at the base.

determines the future developmental pattern. The orientation signal might be one or more chemical gradients which change as the number of nodes increases, and at a critical value of the concentration of the chemicals, the meristem cells alter their gene expression so that floral development ensues. This idea is supported by the work of Schwabe and Al-Doori<sup>6</sup> who proposed that a gibberellic acid (GA) gradient in the black currant (*Ribes nigrum*) may be responsible for the flowering response of axillary meristems. They have shown that the ability of an axillary meristem to flower in short-day photoperiods is directly related to its position on the main axis, and that this response is correlated with the GA gradient.

This regulation of pattern development may be analogous to a model of animal pattern development, where differential gene expression is thought to be intimately related to the orientation of a cell within a developing structure or organism, and that this orientation involves the reception and interpretation of a positional information signal<sup>7</sup>. Although the model has not often been used in studies of pattern development in plants<sup>1,7</sup>, positionally-related development of shoots and leaves has been observed in numerous plants<sup>4,5,8,9</sup>. These observations and the above results suggest that the use of a positional information model could introduce new ways of examining and interpreting developmental patterns in plant systems.

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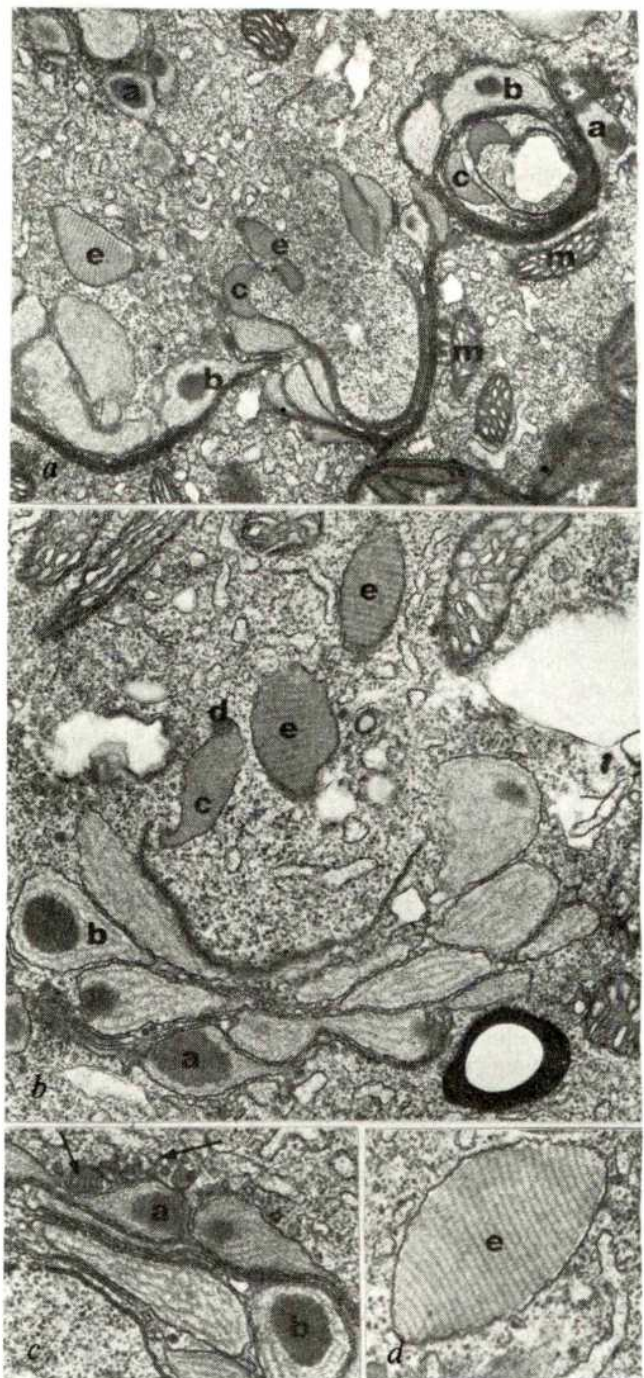


## Unique Golgi apparatus and vesicle formation in a red alga

THE morphology of the Golgi apparatus, as well as the kind and quantity of Golgi-derived vesicles, has been observed to change considerably during carposporogenesis (production of diploid reproductive spores<sup>1</sup>) in red algae<sup>2,3</sup>. In *Polysiphonia* the Golgi is initially active in the production of vesicles destined for wall formation (unpublished), the same function as reported in other red algae<sup>2,3</sup>. At this stage in early development, crystalline structures termed "striated vesicles" have been observed to form within rough endoplasmic reticulum (ER)<sup>2,4</sup>. As carpospore development proceeds the striated vesicles diminish in size and eventually disappear (unpublished). Coinciding with this disappearance is a unique change in the form and function of the Golgi apparatus. The striated vesicles are replaced in the cytoplasm by Golgi-derived vesicles with a crystalline content, their structure and periodicity being different from the striated vesicles. The Golgi apparatus gradually differentiates into its new role, and is not replaced by a new system. Golgi with a similar morphology and function have not been observed before for any cell.

Sexual plants of *Polysiphonia novae-angliae* Taylor were collected in July 1972, at Naushon Island, and in June 1973, at West Falmouth, Massachusetts. The methods used to prepare for electron microscopy have been described before<sup>4</sup>. Figure 1 shows completely differentiated Golgi active in the formation of vesicles with a crystalline content. Mitochondria (m, Fig. 1a) are observed at the base of the forming Golgi face, a situation which is typical throughout carpospore differentiation<sup>2,3</sup>. Proximal cisternae arise from the fusion of ER-derived vesicles which contain a dark osmiophilic material (arrows, Fig. 1c). The periphery of proximal cisternae are swollen with dark osmiophilic regions surrounded by a less dense material (a, Fig. 1a-c). As cisternae mature and are displaced towards the distal Golgi face, the material surrounding the dark osmiophilic regions becomes fibrous with a distinctive pattern and spacing (b, Fig. 1a-c). The osmiophilic regions are not continuous, but somewhat spherical and periodically spaced within a single cisterna. Therefore, they may appear absent in some sections. The fibrous structure gives way to a dark grey, uniformly stained vesicle at the distal pole (c, Fig. 1a and b). The osmiophilic regions then disappear (d, Fig. 1b) as the cut off vesicles take on a fibrous crystalline pattern (e, Fig. 1a, b and d). Ribosomes are not observed to be associated with the membrane surrounding these Golgi-derived vesicles. The vesicles remain in the cytoplasm for a short period in development and then gradually disappear. At this time the Golgi apparatus once again changes in form and function, producing yet another kind of vesicle (unpublished).

It is interesting that the change in Golgi function reported here corresponds exactly with the disappearance of the striated vesicles formed by the rough ER. There may be some correlation between this disappearance and the concurring Golgi differentiation. The dark osmiophilic regions seen in developing cisternae arise during the formation of cisternae, the material having the same appearance as the contents of the fusing ER vesicles at the proximal face. As the periphery of the maturing cisternae enlarge, most contents undergo structural change whereas the osmiophilic regions seem unaltered until near maturity. Contents therefore seem segregated in individual developing cisternae, one transferred from the ER, the other synthesised within the Golgi. Only in mature vesicles do the contents become uniform. Several different types of



**Fig. 1** a, Mature Golgi apparatus active in the production of crystalline vesicles. Mitochondria (m) are located at the base of the proximal Golgi face when sectioned through that region. a, Initial stage in maturation of cisternae. Note the dark osmiophilic region surrounded by a less dense material. b, Stage in maturation of cisternae. Osmiophilic regions are surrounded by a fibrous material. c, Vesicle just before crystalline structure arises. d, Osmiophilic region disappearing in mature vesicle. e, Separated vesicles with crystalline structure. ( $\times 15,390$ ). b, Glancing section of mature Golgi showing the different stages of cisternal development ( $\times 27,060$ ). c, ER-derived vesicles (arrows) active in cisterna formation ( $\times 25,080$ ). d, Structure of crystalline vesicle ( $\times 31,900$ ).

secretory cells are known to use the Golgi apparatus for the concentration and transport of protein to the exterior of the cell<sup>5-7</sup>. The combination of protein with other products during this process has likewise been suggested<sup>6,7</sup>. Crystalline structures described within other cells have consistently been determined to be at least partially proteinaceous<sup>9,10</sup>. This is the first observation of Golgi-derived vesicles with a crystalline content; since these vesicles are



retained in the cytoplasm only for a short period during development, evidence suggests that the Golgi has a role in the transformation and turnover of protein within differentiating carpospores. Golgi apparatus with a similar fine structure and function have not been reported before.

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## Possible regeneration of $\gamma$ -aminobutyric acid-containing fibres into irides transplanted into the central nervous system

IRIDES transplanted into the central nervous system (CNS) of the rat provide a good substrate for regenerative ingrowth of central noradrenergic and cholinergic fibres to 'reinnervate' the denervated iris<sup>1-3</sup>. Fluorescence histochemistry or acetylcholinesterase staining reveals that the regenerating noradrenergic or cholinergic fibres will produce an innervation that closely mimics the original noradrenergic or cholinergic innervation<sup>1-3</sup>. Physiological experiments<sup>4</sup> on portal vein transplants indicate that functional adrenergic and cholinergic terminals are formed, and it seems likely that functional neuromuscular junctions are also re-established in the iris transplants. In addition to supporting the ingrowth of noradrenergic and cholinergic fibres, fluorescence histochemistry<sup>5</sup> reveals that 5-hydroxytryptamine- and dopamine-containing fibres will also grow into iris transplants, albeit less extensively than noradrenergic fibres. This ability of the iris to support regenerative ingrowth by fibres not entirely appropriate for normal reinnervation suggested to us that the iris might also support regenerative ingrowth by fibres containing putative amino acid transmitters.

As a possible marker for  $\gamma$ -aminobutyric acid (GABA) terminals we choose to measure the GABA synthetic enzyme, glutamic acid decarboxylase (GAD). GAD is located in nerve terminals and is concentrated in areas of the brain where GABA is believed to act as a neurotransmitter<sup>6,7</sup>. We demonstrate here that irides transplanted into the adult rat CNS show increasing levels of glutamic acid decarboxylase consistent with the ingrowth of GAD containing fibres. In addition, using a microdansylation assay<sup>8</sup> we have measured the more concentrated amino acids in the transplants and in normal and sympathectomised irides, and found substantial increases in levels of GABA, glutamate and glutamine in the transplants, which would be consistent with regenerative ingrowth of fibres

containing relatively high concentrations of these amino acids.

The experiments were carried out on adult female Sprague-Dawley rats (180-200 g body weight). Autologous transplantations of the iris were made in two brain sites; one in the hippocampal fimbria and the rostral hippocampus (CA3), as described earlier<sup>3</sup>, and a second in the head of the caudate nucleus (unpublished). In both sites the transplants survive well with little bleeding and become reinnervated by acetylcholinesterase-containing fibres, and in the caudate site by dopamine-containing fibres as well. All animals were subjected to cranial sympathectomy through bilateral extirpation of the superior cervical ganglia. In addition, as a control site, irides were transplanted to the anterior eye chamber<sup>3</sup>. In one experiment irides transplanted to the caudate nucleus 21 d earlier were transplanted to the anterior eye chamber. Here, they were allowed to survive for another 4 d. GAD levels were initially measured by <sup>14</sup>CO<sub>2</sub> evolution<sup>9,10</sup> in the presence of 0.5% Triton X-100, a concentration recently shown<sup>11</sup> to reduce the possible formation of <sup>14</sup>CO<sub>2</sub> by glutamate entering the tricarboxylic acid cycle as a ketoglutarate.

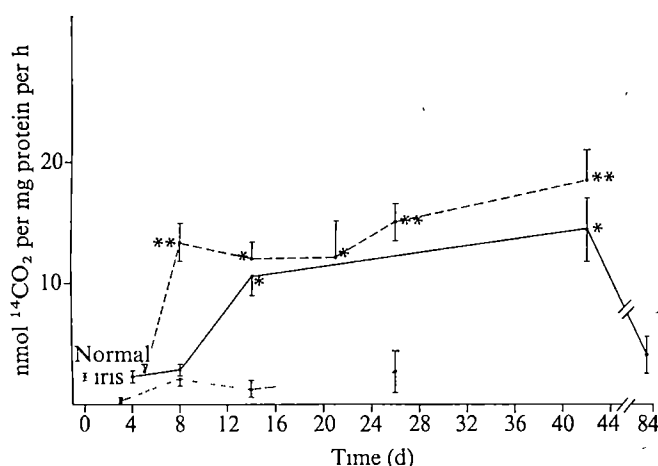
The results are summarised in Fig. 1, and the assay conditions given in more detail in Table 1. In both the caudate nucleus and the fimbrial-hippocampal site there was a marked increase in GAD in the transplants, its appearance following a time development similar to that of the in-

**Table 1** Comparison of glutamic acid decarboxylase activity in normal and sympathectomised irides, and in irides transplanted to caudate and fimbrial sites

	GABA production (nmol per mg protein per h)	<sup>14</sup> CO <sub>2</sub> production (nmol per mg protein per h)
Normal iris	< 0.080 (3)	2.38 ± 0.6 (12)
Sympathectomised iris	< 0.080 (3)	3.38 ± 0.7 (6)
Iris transplant to anterior eye chamber (26 d after transplantation)	—	2.7 ± 1.7 (6)
Caudate transplant (21 d GABA production, or 26 d <sup>14</sup> CO <sub>2</sub> production)	4.47 ± 1.16 (5)**	14.9 ± 1.2 (6)*
Fimbrial transplant (21 d GABA production or 14 d <sup>14</sup> CO <sub>2</sub> production)	4.23 ± 0.67 (3)**	10.5 ± 1.6 (6)*

Values are means ± s.d. Means were derived by Student's *t* test (two tailed) \**P* < 0.05, \*\**P* < 0.01. The figures in parentheses are numbers of replicates. <sup>14</sup>CO<sub>2</sub> production was measured using the micromodification of the method of Albers and Brady<sup>9</sup> described by Fonnum *et al.*<sup>10</sup>. DL-1-<sup>14</sup>C-glutamate (58 mCi mmol<sup>-1</sup>, New England Nuclear) was used as substrate. 2 µl of an iris homogenate (≈ 1.0 mg per 20 µl buffer) was incubated for 1 h with a substrate containing final concentrations: L-1-<sup>14</sup>C-glutamate, 0.30 mM; K-L-glutamate, 4.03 mM; potassium phosphate buffer (pH 6.5), 40.3 mM; pyridoxal-5-phosphate, 0.260 mM; dithiothreitol, 2.38 mM and 0.5% Triton X-100. <sup>14</sup>CO<sub>2</sub> was evolved by adding a drop of 1 M sulphuric acid and <sup>14</sup>CO<sub>2</sub> collected in a separate microtube containing 50 µl solene (Packard). <sup>14</sup>C-GABA production was measured using L-(U)-<sup>14</sup>C-glutamate (290 mCi mmol<sup>-1</sup>, Radiochemical Centre, Amersham). 10 µl of iris homogenate (≈ 1.0 mg per 10 µl) was incubated for 3 h with a substrate containing final concentrations: L-(U)-<sup>14</sup>C-glutamate, 0.17 mM; K-L-glutamate, 3.4 mM; potassium phosphate buffer (pH 6.5), 34 mM; pyridoxal-5-phosphate, 0.44 mM; dithiothreitol, 3.9 mM; 0.25% Triton X-100 and 1.35 µM amino-oxycetic acid. The reaction was stopped by addition of 0.96 N perchloric acid, the protein centrifuged down and the pellet used for protein determination by the method of Lowry *et al.*<sup>13</sup>. The supernatant (20 µl) after neutralisation with 2 M potassium carbonate was reacted with 20 µl dansyl chloride (2 mg ml<sup>-1</sup>) (British Drug Houses) for 2 h, evaporated to dryness and taken up in 20 µl acetone-acetic acid (3:2, v/v). The total volume was applied to full size polyamide plates and dansyl GABA separated by chromatography as described by Joseph and Haliday<sup>7</sup>. <sup>14</sup>C-GABA standards were taken through the procedure to correct for recovery. There was no spread of the labelled substrate (dansyl glutamate) to contaminate the dansyl GABA spot and no significant radioactivity was detected outside these spots. The limit of sensitivity of this assay, double the blank value, was equivalent to ≈ 0.25 pmol.





**Fig. 1** Glutamic acid decarboxylase activity of irides transplanted to a fimbrial-hippocampal site (—), a caudate site (---) and an ocular site (.....). Values are means  $\pm$   $\sigma$  of at least five separate determinations at each time point. Differences from normal iris were compared using Student's *t* test (two tailed) \**P* < 0.05, \*\**P* < 0.01.

growth of regenerating monoaminergic and cholinergic fibres (see refs 2, 3 and unpublished observations). The increase in GAD cannot be a response to denervation alone, as sympathectomy (Table 1) does not significantly alter the level of GAD in the iris. Moreover, the increase in GAD does not occur after transplantation of the iris to the anterior eye chamber (Fig. 1). In fact, there seemed to be a temporary decrease in GAD levels suggesting that some of the GAD activity of the normal iris might reside in the muscle coat which, during the early stages after transplantation, undergoes degeneration<sup>12</sup>.

The reduction in <sup>14</sup>CO<sub>2</sub> production seen in the 4-d-old irides transplanted to the anterior eye chamber indicates the problems of correlating <sup>14</sup>CO<sub>2</sub> evolution with glutamic acid decarboxylase activity. For this reason we felt it necessary to also measure GAD by GABA production from L-(U)-<sup>14</sup>C-glutamate (Table 1). The results show that in both the caudate and the fimbrial-hippocampal sites definite GABA formation occurred in the transplanted irides, whereas GAD activity was low or absent in the normal or sympathectomised irides. Measured in this way there was a 100-fold increase in GAD activity in the CNS implants over the normal or sympathectomised irides. The

average GAD activity of the transplants measured by <sup>14</sup>C-GABA formation was, however, less than that observed with the CO<sub>2</sub> evolution assay. These differences were not great and could readily be explained by the normal variations in GAD activity of the transplants and by the fact that the GAD assay is undoubtedly no longer linear after 3 h incubation. Three hours was chosen, to ensure sufficient counts in the dansyl-GABA spot; the apparent GAD activity would be greater if the incubation were performed over 1 h. The very small amount of GABA formed in the normal iris (in some cases no formation was detected) confirms our suggestion that basal level of GAD activity in the normal iris as measured by <sup>14</sup>CO<sub>2</sub> production is not related to GABA formation but rather probably represents glutamate metabolism by other pathways to yield <sup>14</sup>CO<sub>2</sub>.

In addition to demonstrating the appearance of significant levels of GAD in the transplanted irides it was of great interest to look at changes in the levels of amino acids in the irides after transplantation to the two brain sites (Table 2). In both the caudate and fimbrial-hippocampal sites there were significant increases in GABA, glutamate and glutamine above the levels in normal or sympathectomised irides. In contrast, the levels of taurine, glycine and aspartate did not change significantly. The level of GABA (2.3–2.5  $\mu$ mol per g protein) correlates well with the level of GAD (20–30  $\mu$ mol per h per g protein) and the ratio of GABA–GAD is similar to that found in the caudate nucleus or hippocampus and other sites believed to contain GABA terminals<sup>14,15</sup>. Glutamine may have an important role in GABA metabolism or ammonia formation<sup>16</sup>. It seems possible, therefore, that the appearance of GABA and glutamine in the central implants both reflect the ingrowth of GABA-ergic fibres. This might be the case with glutamate as well, but it also seems possible that glutamate could be found in a separate, glutamate-rich fibre type (see refs 15, 17–20).

These various results are consistent with a growth into the transplanted irides of GABA-synthesising and GABA-containing elements from the brain tissue surrounding the transplantation site. There are two possible, though unlikely, objections to this interpretation. First, the increased GAD and amino acid levels could arise from a growth of fragments of retinal tissue (which contain both GAD and GABA) which might be attached to the iris transplant. This can be excluded, however, since adult retinal tissue does not survive transplantation to the CNS sites used

**Table 2** Amino acid levels in transplants from fimbrial-hippocampal and caudate sites (21 d post transplantation)

Amino acid	Normal iris ( <i>n</i> = 3)	Sympathectomised iris ( <i>n</i> = 3)	Caudate transplant ( <i>n</i> = 5)	Fimbrial-hippocampal transplant ( <i>n</i> = 4)	Caudate nucleus ( <i>n</i> = 3)	Hippocampus ( <i>n</i> = 4)
$\gamma$ -Aminobutyric acid	0.02 $\pm$ 0.005	0.030 $\pm$ 0.014	0.23 $\pm$ 0.012*†	0.25 $\pm$ 0.042†*	1.85 $\pm$ 0.24	1.76 $\pm$ 0.13
Glycine	0.35 $\pm$ 0.052	0.52 $\pm$ 0.03*	0.50 $\pm$ 0.03	0.61 $\pm$ 0.10	0.36 $\pm$ 0.02	0.42 $\pm$ 0.021
Glutamine	0.307 $\pm$ 0.077	0.373 $\pm$ 0.13	2.68 $\pm$ 0.35†*	3.35 $\pm$ 1.24*	4.59 $\pm$ 0.29	4.90 $\pm$ 0.55
Glutamate	0.501 $\pm$ 0.022	0.68 $\pm$ 0.09	1.26 $\pm$ 0.078†*	1.40 $\pm$ 0.15†*	5.08 $\pm$ 0.126	6.16 $\pm$ 0.35
Aspartate	0.71 $\pm$ 0.037	0.473 $\pm$ 0.09	0.59 $\pm$ 0.083	0.97 $\pm$ 0.34	0.97 $\pm$ 0.16	0.73 $\pm$ 0.11
Taurine	4.01 $\pm$ 0.87	4.22 $\pm$ 0.54	3.41 $\pm$ 0.4	4.0 $\pm$ 0.87	5.82 $\pm$ 0.25	4.75 $\pm$ 0.28

Levels in transplants are compared with amino acid levels in the normal and sympathectomised iris and with levels in the caudate nucleus and hippocampus. Units are  $\mu$ mol per 100 mg protein.

Means were compared with Student's *t* test (two tailed) \**P* < 0.05, †*P* < 0.01, ‡*P* < 0.001. ( ) indicate significance if normal and sympathectomised iris data are pooled for comparison with fimbrial and caudate transplants. Amino acid levels were determined using the microdansylation assay for amino acids described in detail by Joseph and Haliday<sup>7</sup>. Because of the small amounts of tissue in the iris samples (0.8–1.5 mg wet weight) the samples were homogenised in 10  $\mu$ l of 0.48 M perchloric acid, and after centrifuging 2  $\mu$ l of the <sup>14</sup>C-amino acid mixture (GABA, glycine, glutamine, glutamate and aspartate, Radiochemical Centre, Amersham) and 2  $\mu$ l 2 M potassium carbonate were added to the supernatant and the sample again centrifuged to remove the precipitated perchlorate. An aliquot of the supernatant (2  $\mu$ l) was reacted with <sup>3</sup>H-dansyl chloride (5.1 mCi  $\mu$ mol<sup>-1</sup>, Radiochemical Centre, Amersham) for 30 min at room temperature after which the sample was evaporated to dryness. The dansylated sample was redissolved in 5  $\mu$ l acetone–acetic acid (3:2, v/v) and 0.3  $\mu$ l applied to small polyamide plates 3.5 cm<sup>2</sup>. The dansylated amino acids were separated using the solvents described by Joseph and Haliday<sup>7</sup>, the spots visualised, cut out and the <sup>3</sup>H–<sup>14</sup>C ratio determined by two-channel liquid scintillation spectrometry. The <sup>3</sup>H–<sup>14</sup>C ratio is independent of recovery and degree of dansylation. Suitable standards and blocks are run simultaneously and the amount of amino acid determined from standard values. Protein content of the pellets was determined by the method of Lowry *et al.*<sup>13</sup>.

**Table 3** Effects of chloride ions (as sodium chloride) on apparent GAD activity in irides transplanted into caudate nucleus (21 d survival), in control caudate nucleus tissue, and in tissue from the pineal gland

	No chloride in incubation mix (GAD)	200 mM chloride in incubation mix (GAD)	Enzyme assayed	Irides transplanted from caudate nucleus (21 d survival) to anterior eye chamber (4 d survival)	Irides transplanted to caudate nucleus (26 d survival)
Irides transplanted to caudate nucleus (21 d survival)	12±3.2	6.1±3.4*	GAD	ND	14.9±1.2
Pineal gland	9.3±1.1	8.9±1.0	ChAT	< 0.14(*)	89±10
Caudate nucleus	223±14	129±21*	AAD	ND	5.9±0.4

The table also shows the effects of retransplantation on GAD activity, choline acetyltransferase activity (ChAT) and aromatic amino acid decarboxylase activity (AAD) in irides transplanted to the caudate nucleus and then retransplanted into the anterior eye chamber of a different rat. For comparison, enzyme levels from iris transplants removed from the caudate nucleus site after 26 d and then assayed for ChAT, AAD and GAD are shown (this paper and Emson, Björklund and Stenevi, unpublished). Values are means ± s.d. Units are nmol per mg protein per h. Means were compared using Student's *t* test (two tailed) on paired or unpaired samples. \**P* < 0.001, (\*) paired *t* test, *P* < 0.001. ND = not detectable.

(unpublished observations), and since identical transplants to the anterior eye chamber (which is a comparable or even better transplantation site than the brain) did not show the increased GAD levels. Second, it might be argued that, in removing the iris transplants from the brain, we are also removing trace amounts of surrounding tissue rich in GAD and amino acids. We were aware of this possibility and paid particular attention during dissection to removing all brain tissue fragments visible under high magnification in the dissection microscope. Random identical specimens were also taken for microscopic analysis showing that the amount of adhering tissue was minimal, at least up to ~ 1 month after transplantation. The amount of tissue adhering would, moreover, need to be fairly substantial to account for the GAD levels observed. The GAD level in the caudate nucleus is 217 μmol per h per g protein<sup>14</sup>, whereas the GAD activity in the best caudate implants was 30–40 μmol per h per g protein. To account for the weight GAD activity in a transplant (~ 0.8 mg wet weight) we would need ~ 100 μg of adhering tissue, an amount we would be most unlikely to fail to detect. It is also obvious that if adhering tissue would account for the observed increases in amino acid levels in the transplants, then the increases should reflect the relative abundance of the various amino acids in the tissue surrounding the transplant. This was not the case (see Table 2).

Thus, it seems likely that the observed increases in enzyme and amino acid levels in the centrally transplanted irides resulted from the growth of tissue elements from the surrounding, lesioned brain tissue. To the extent that GAD and the GABA-GAD ratio can be taken as good markers for GABA-containing terminals in the brain, the GAD and GABA levels in the implanted tissue should provide good measures of the sprouting of regenerating GABA-ergic fibres into the implant from the lesioned brain tissue. The likely origin of such sprouting GABA- and GAD-containing fibres would in both the caudate and the hippocampal sites seem to be the local GABA-ergic interneurons which have been demonstrated in these brain regions<sup>21,22</sup>.

The most obvious difficulty in the interpretation of the present findings is to evaluate the contribution of glial cells to the observed increases in enzyme and amino acid levels in the transplanted irides. The strong increase in glutamine in the transplants might be an indication for this, since this amino acid may be relatively concentrated in glial cells<sup>16</sup>. Also GAD is known to occur in glial cells<sup>16</sup>, and we could not exclude the possibility that the GAD (as well as the GABA and glutamate) we measure is located at least partly in glial cells. To obtain further evidence of the neuronal localisation of the GAD activity, we carried out an experiment designed to effect a denervation of the reinnervated transplant. Irides were transplanted to the caudate nucleus as above, and allowed to survive 21 d.

After 21 d, when the GAD levels in the transplant (as measured by the CO<sub>2</sub> evolution assay) were near maximal, the animals were killed, and the transplants dissected out from the caudate nucleus. The iris was then retransplanted into the anterior eye chamber of another rat. Here, the iris remained for 4 more days—a survival time that would allow for the degeneration of fibres that had previously regenerated into the transplant—and was then assayed for GAD, choline acetyltransferase (ChAT) and aromatic amino acid decarboxylase (AAD). As shown in Table 3 there was a complete or almost complete loss of all three enzymes. These results are consistent with a degeneration of cholinergic, dopaminergic and GABA containing terminals that had grown into the transplant while being in the caudate nucleus. A further pointer to the probable neuronal origin of the GAD in the transplant is shown by its response to Cl<sup>-</sup> ions<sup>23</sup>. GAD activity in the pineal gland, which is believed to be exclusively glial in origin, was not significantly reduced by the addition of Cl<sup>-</sup> ions, whereas the activity in the transplants—like that in the caudate nucleus—was reduced some 50% (*P* < 0.001).

Very little is known about the regeneration of GABA neurons. Baxter and Torralba<sup>24,25</sup> have provided some evidence for regeneration of GABA neurons in insects. Recent studies (see refs 2 and 3) have shown that many non-myelinated axon systems in the adult mammalian CNS have a high capacity for regenerative sprouting. The present results are compatible with the idea that this is the case also for non-myelinated GABA-ergic interneurons in the hippocampus and the caudate nucleus. If this is so our observations constitute the first direct evidence for regeneration of GABA neurones in the adult mammalian CNS.

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## Absence of transient tritanopia after adaptation to very intense yellow light

STILES reported<sup>1</sup> that after the eye had been adapted to light of high intensity and long wavelength, its sensitivity to violet test flashes did not recover according to the familiar dark-adaptation curve. Instead the threshold intensity for detecting short-wavelength flashes increased when the adapting field was turned off, and the peak sensitivity of the eye lay at long wavelengths. Yet it was by using very similar conditions that Auerbach and Wald<sup>2</sup> isolated a photoreceptor with peak sensitivity in the violet. This contradiction has not been resolved. Transient tritanopia (as we have provisionally termed the loss of sensitivity found by Stiles) has been reported by others<sup>3-6</sup> and an electrophysiological counterpart has been described<sup>7</sup>, but equally there are findings that resemble the contrasting result of Auerbach and Wald<sup>8,9</sup>. Attempting to resolve this contradiction, we have discovered a second surprising property of transient tritanopia.

Although our previous measurements had shown that the phenomenon occurred over a large range of adaptation intensities, and became more marked as the intensity of the adaptation field increased<sup>10</sup>, we had not used fields quite as intense as the 6.0–7.0 log trolands used by those who had found that adaptation left the eye blue-sensitive, rather than blue-blind. Moreover, the suprathreshold hue-shift described previously<sup>5</sup> does not occur if the adapting field is too bright. Stiles worked with a red field of 4.3 log trolands. We have therefore measured sensitivity to short wavelengths after adaptation to retinal illuminances that varied over the range 1.1–6.0 log trolands.

Blue (445 nm) test flashes were presented 400 ms after extinction of a yellow adapting field. Target flashes subtended 1° of visual angle, were presented to the fovea and lasted 15 ms. To secure very intense adapting fields, the interference filter previously used in the adapting beam<sup>5</sup> was replaced by a gelatin spectral filter (Ilford No. 626) which has a peak transmission at 575 nm and a bandwidth at half height of 35 nm. The test flashes and the adapting field were concentric and were presented in Maxwellian view. Fixation was guided by four illuminated points that were arranged in a diamond with a vertical and horizontal separation of 3° and were adjusted in intensity so that they were just clearly visible against a particular adaptation field. For 3 s every 18 s the adapting field was interrupted by a dark interval and a single target flash was presented 400 ms after the field had been turned off. (We chose 400 ms because at this delay the brief increase in threshold found for white light<sup>11</sup> has ceased, but transient tritanopia is still marked<sup>1</sup>.) The experiment was under computer

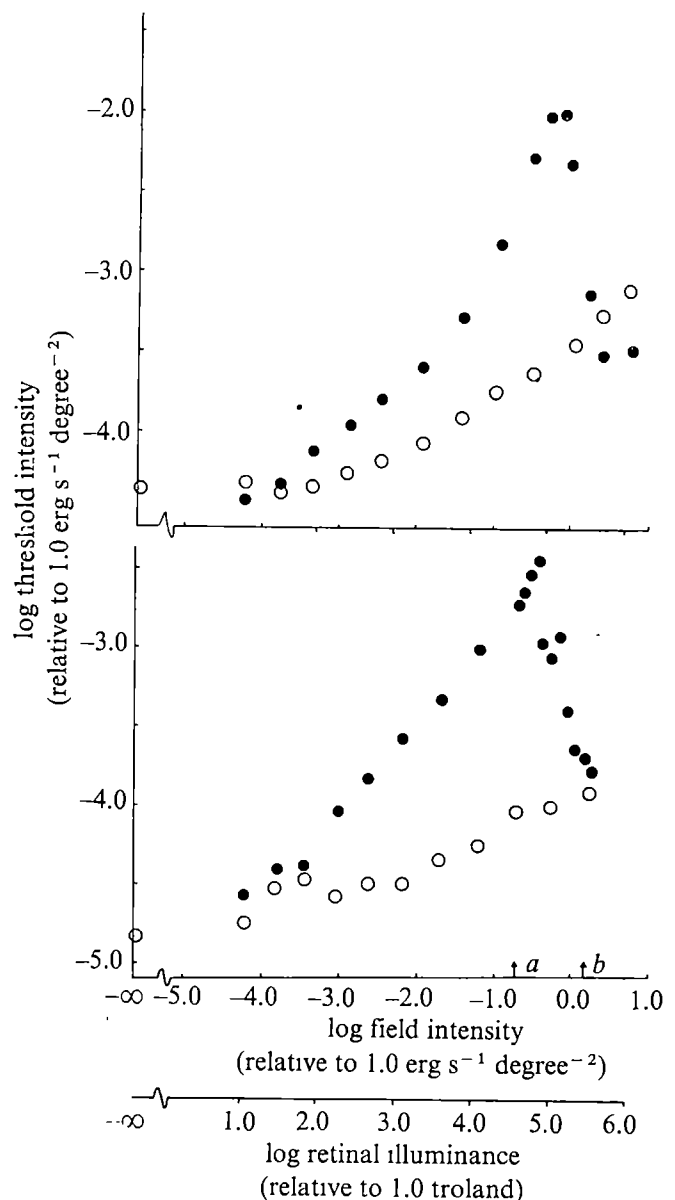


Fig. 1 Ordinate, threshold intensity at which blue target detected; abscissa, intensity of adapting field. ○, Log threshold intensity for detecting blue flash when a steady yellow field is present (conventional increment-threshold function); ●, threshold 400 ms after adapting field has been turned off (means of two runs). Field intensity marked *a* corresponds to adaptation level that gave results of Fig. 2, that marked *b* corresponds to level that gave results of Fig. 3. Observers: I.D.M. (upper panel), P.G.P. (lower panel).

control and the threshold for detecting the flash on 50% of trials was measured by a double staircase procedure<sup>12</sup>. No data were gathered until the field had been cycling on and off for 4 min. Each estimate of the threshold was based on 50 trials.

Figure 1 shows results for two observers. When the field was present and was between 2.0 and 4.5 log trolands the mechanism responsible for detection was that termed  $\pi_1$  by Stiles: note that transient tritanopia occurred even for fields that did not significantly adapt  $\pi_1$  in the steady state. As the field increased to 5.0 log trolands there was an increasing difference between the threshold measured in the steady state and that measured 400 ms after the adapting field had been turned off. As Fig. 2 shows, however, the mechanism mediating detection of the flashes after extinction of a field of 4.58 log trolands had the spectral sensitivity of  $\pi_1$ , Stiles' green-sensitive mechanism, and thus the true extent of the suppression of the blue cone mechanism is concealed in this range of adaptation illu-

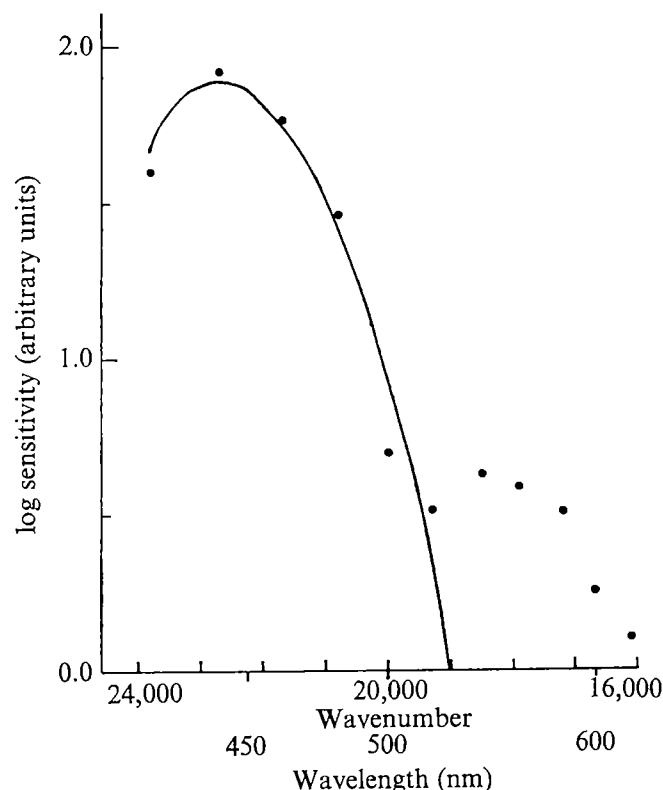
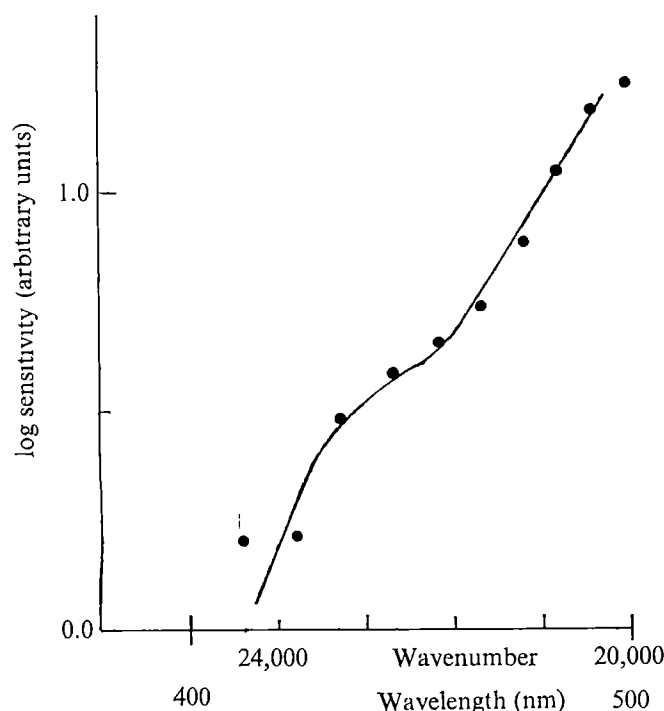
minance the theoretical threshold for  $\pi_1$  must lie above the solid circles of Fig. 1.

The most unexpected results were found at an adaptation illuminance of about 5.0 log trolands. As the field that was turned off became brighter, the threshold fell precipitously. In other words, the eye became more sensitive to short wavelengths as the luminance of an adapting field was increased. Moreover, it was now a blue-sensitive mechanism that became responsible for detection of the 445-nm targets. Figure 3 shows the spectral sensitivity of the eye 400 ms after a field of 5.47 log trolands has been turned off; this can be compared with Auerbach and Wald's Fig. 4 (ref. 2) and contrasted with our Fig. 2.

It now seems clear why there are two apparently contradictory reports in the literature. Stiles<sup>1</sup> used an adapting field of 4.3 log trolands whereas those who did not report transient tritanopia<sup>2,8,9</sup> used more intense fields. (The experiment of Norren and Padmos<sup>8</sup> has a second relevant feature: an initial white bleaching field was replaced by an intense long wavelength field designed to preserve the light adaptation of the red- and green-sensitive mechanism while the recovery of the blue-sensitive cones was followed. If transient tritanopia arises when a recovering long-wavelength mechanism inhibits or masks the signals from the blue-sensitive cones<sup>5</sup>, we should not expect the phenomenon in the conditions used by Norren and Padmos.) A difficulty for this resolution is posed by the results of Das<sup>3</sup>, who reported transient tritanopia after adaptation to a field of " $1.86 \times 10^6$  trolands"; but there is a discrepancy between this value and the abscissa of his Fig. 1.

The recovery of sensitivity at adaptation illuminances above 5.0 log trolands is sudden: in the case of both observers the function relating threshold to adaptation illuminance has a maximum slope steeper than  $-2$ . Although little is known about this phenomenon, the recovery looks like a disinhibition and a working hypothesis might be that the point at which the threshold collapses represents the retinal illuminance at which some mech-

**Fig. 2** Sensitivity of eye to short wavelengths 400 ms after a yellow adapting field of 4.58 log trolands has been turned off. Ordinate, reciprocal of intensity required to detect a test flash of varying wavelength. Conditions as for main experiment, except that several test wavelengths and only one adaptation intensity were used. Observer: P.G.P. Solid curve, Stiles' field sensitivity function for  $\pi_4$  displaced vertically to give best fit to experimental points.



**Fig. 3** Spectral sensitivity of eye 400 ms after extinction of a yellow field of 5.47 log trolands. Ordinate, reciprocal of intensity required to detect a test flash of varying wavelength. Observer: P.G.P. Solid function fitted to short wavelength points is Stiles' field sensitivity function for  $\pi_1$ .

anism more sensitive to the long wavelength field has become too bleached, or otherwise too refractory, to inhibit or mask the blue-sensitive mechanism during early dark adaptation. Thus our results add to the suspicion<sup>8</sup> that transient tritanopia represents not a direct effect of the extinction of the field on the blue cones but the inhibition or masking of the signals of the blue cones by a mechanism with different spectral sensitivity. The collapse of transient tritanopia in Fig. 1 occurs at approximately the field intensity at which the mechanism termed  $\pi_3$  by Stiles replaces  $\pi_1$  in the normal increment-threshold function: the inhibition seen in transient tritanopia is possibly related to an inhibition that marks the difference between  $\pi_1$  and  $\pi_3$  in the steady state. ' $\pi_1$ ' may represent those conditions in which the gain of the blue-sensitive mechanism is controlled by a long wavelength mechanism as well as by quanta absorbed directly by the blue-sensitive pigment.

Fields of  $\sim 10^8$  trolands bleach a substantial fraction of the rhodopsin in the retina within 30 s (ref. 13) and prolonged observation of such fields (for example, in making the measurements of Fig. 3) left us with after-images that lasted for as long as 7 d. Stiles has told us that he noticed such after-images when working in similar conditions, and Brindley<sup>14</sup> reported an after-image lasting 8 months, which was the cumulative result of repeated adaptation to high intensities. Although we can ourselves detect no permanent damage, we recommend caution in repeating our measurements: only one eye should be used and the possible dangers should be explained to all subjects.

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## Restoration of sensitivity of cultured hepatoma cells to cyclic nucleotides shows permissive effect of dexamethasone

CULTURED rat hepatoma cell lines H-35, HTC and RLC respond to glucocorticoid hormones and have revealed details of how these steroids regulate the activity of tyrosine aminotransferase (TAT) that could not have been learned using intact liver<sup>1–4</sup>. Unfortunately, the apparent lack of other regulatory mechanisms normally present in liver often limits the usefulness of these lines. For example, although cyclic AMP or its dibutyryl derivative reproducibly cause a several-fold induction of TAT in adult<sup>5</sup> and foetal rat liver<sup>6</sup> and in the H-35 (refs 7 and 8) and RLC<sup>9</sup> hepatoma cell lines, these compounds have generally been found to be ineffective inducers of HTC cell tyrosine aminotransferase<sup>8–10</sup>. Taken with the demonstration that HTC cells have marginally detectable levels of adenylate cyclase and cyclic AMP, this insensitivity to cyclic nucleotides led to the suggestion that glucocorticoid induction does not depend on cyclic AMP<sup>10</sup>. The following evidence suggests that the converse may not be true: TAT induction by dibutyryl cyclic AMP (db cyclic AMP) in adult rat liver is reduced by adrenalectomy<sup>11</sup>; combinations of db cyclic AMP and steroids result in synergistic induction in rat liver and liver organ culture<sup>5</sup>; and cortisol increases the absolute degree of induction of TAT by db cyclic AMP in some responsive hepatoma cell lines<sup>8,12</sup>. This putative inter-relationship could be clarified if a cell line, otherwise unresponsive to cyclic nucleotides, could be made to respond to these agents by adrenal steroid hormones. This report describes such an accomplishment and suggests that HTC cells are a model system for studies of the permissive effects of glucocorticoid hormones.

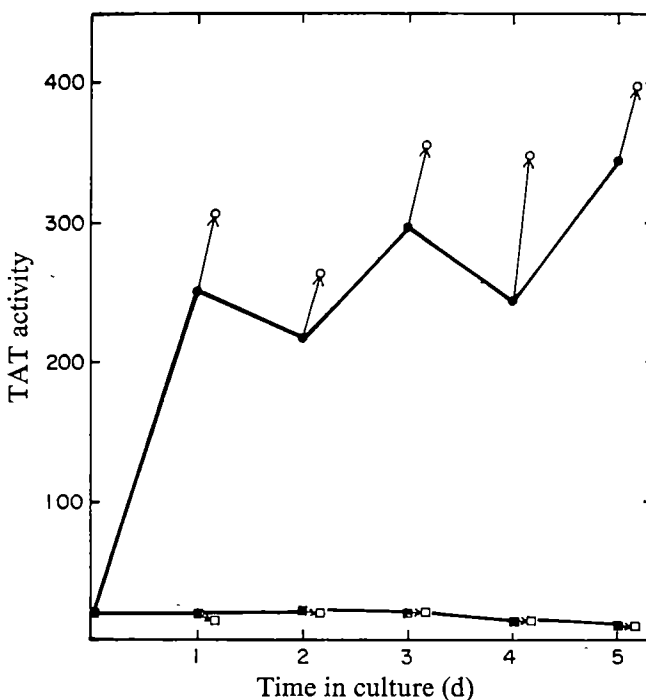
Addition of 1 mM db cyclic AMP to HTC cells grown in suspension for 1–5 d in modified Swim's S77 medium<sup>9</sup> results in no increase in TAT activity over the basal value (Fig. 1). This lack of induction<sup>10</sup> has repeatedly been demonstrated in our line of HTC cells and those of others<sup>8,9</sup> and thus seems to represent a stable trait. Two groups have found a small (35–50%) increase in TAT activity when db cyclic AMP was added to monolayer cultures of HTC cells<sup>12,13</sup>. If significant, this induction may reflect different culture conditions or variant strains, and resolution of this difference awaits further experimentation. Supplementation of the standard medium with 5  $\mu$ M dexamethasone results in 15- to 20-fold induction of HTC cell TAT, a process which has been shown to be attributable to an increased rate of synthesis of the enzyme<sup>3</sup>. Addition of 1 mM db cyclic AMP to these steroid-treated cells results in a further increase in TAT-specific activity (Fig. 1). This 'reinduction'—so-called because it is again possible to elicit a response associated with normal hepatocytes, and to differentiate it from superinduction<sup>4</sup> and deinduction<sup>14</sup> which define specific aspects of TAT regulation—is evident 24 h after addition of dexamethasone to the medium, persists for at least 5 d if hormone is kept in the

medium, and has been consistently demonstrated for the past 18 months. The extent of reinduction, greatest on day 4 of this experiment, represents an increase in activity of 100 mU per mg protein, and thus is equivalent to a tenfold induction over the basal value of 10 mU per mg protein. Reinduction requires specific nucleotides. Although *N*<sup>6</sup>-monobutyryl cyclic AMP is also effective, cyclic AMP, cyclic GMP, adenosine and sodium butyrate do not work (data not shown).

The kinetics of reinduction are shown in Fig. 2. TAT activity begins to increase 60–90 min after addition of db cyclic AMP and maximal levels are achieved between 4–6 h. This time course resembles that seen when db cyclic AMP is added to RLC cells, a cell line in which we have found TAT to be inducible in the absence of glucocorticoid hormones<sup>9</sup>.

To investigate whether reinduction of TAT in HTC cells requires protein synthesis, 0.1 mM cycloheximide was added 30 min before addition of db cyclic AMP. This concentration, which inhibits protein synthesis by 95% and prevents steroid induction if added before the hormone<sup>2</sup>, also completely blocks the additional induction of TAT seen when 1 mM db cyclic AMP is added to HTC cells grown in medium supplemented with dexamethasone (Table 1). Thus continued protein synthesis is required for the expression of reinduction. Preliminary experiments using a highly specific anti-TAT antibody suggest that there is a parallel increase in TAT antigenic and catalytic activity with reinduction, and that there is a specific increase in the rate of synthesis of the enzyme. Reinduction thus seems

**Fig. 1** Restoration of sensitivity of HTC cells to db cyclic AMP. HTC cells were grown in a modified Swim's S77 medium<sup>9</sup> supplemented with 5% foetal calf and 5% calf sera heat inactivated at 56 °C for 30 min, or in this medium containing 5  $\mu$ M dexamethasone (gift of Merck, Sharpe and Dohme), and were diluted with the appropriate medium every other day to maintain cells in log growth. Aliquots of cells were removed from each culture daily and adjusted to a concentration of 500,000 cells ml<sup>-1</sup>, then db cyclic AMP (Boehringer-Mannheim) was added to a final concentration of 1 mM. Four hours later TAT specific activity, equivalent to nmol *p*-hydroxyphenylpyruvate formed per mg protein per min, was determined<sup>9</sup>. Cells grown in absence of dexamethasone before (■) and after (→□) addition of db cyclic AMP; cells grown in the presence of dexamethasone before (●) and after (→○) addition of the nucleotide. Daily variation in TAT activity in cells grown in dexamethasone probably reflects induction from the serum in fresh medium<sup>22</sup>.



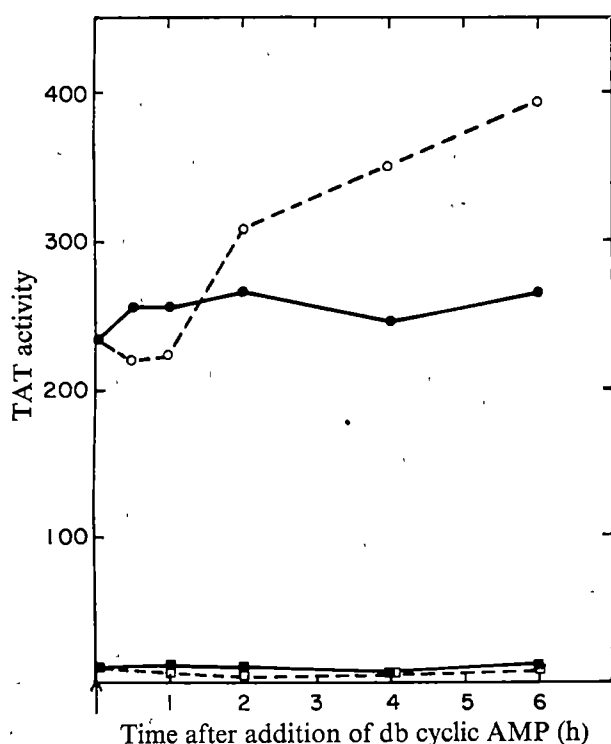


Fig. 2 Time curve of reinduction. HTC cells taken from cultures on day 4 of the experiment described in Fig. 1 were resuspended at a concentration of 500,000 cells  $\text{ml}^{-1}$  in modified S77 medium  $\pm$  dexamethasone. Dibutyl cyclic AMP (1 mM) was added to half of each culture and samples were taken for determination of TAT activity at times indicated on abscissa. Control cells without (□) or with (■) db cyclic AMP; dexamethasone-treated cells without (○) or with (●) nucleotide.

to involve mechanisms similar to the induction of TAT by steroid hormones<sup>3</sup>.

Shortly after glucocorticoid hormones were discovered, Ingle proposed that they have a general role in enabling maximal metabolic rates of processes essential to homeostasis<sup>15</sup>. Subsequently several permissive effects involving a variety of processes have been elucidated. Among these effects are those of adrenaline on gluconeogenesis<sup>16</sup>, glycogenolysis<sup>17</sup> and lipolysis<sup>18</sup>, of mammary tumour virus production<sup>19</sup>, of glucagon on amino acid transport into isolated hepatocytes<sup>20</sup>, and of growth factor stimulation of fibroblast proliferation<sup>21</sup>. The reinduction of TAT by db cyclic AMP in the strain of HTC cells used in this study is of interest because the steroid is not just enhancing a response (as in the other examples of TAT induction<sup>5,8,11,12</sup>) but rather is absolutely required. Although little is known about the mechanism(s) of any permissive effect, a great deal is known about the regulation of TAT. A well defined cultured cell system is thus available for investigating this important action of glucocorticoid hormones.

The lack of regulatory mechanism in cultured cells is

Table 1 TAT activity (nmol per mg protein per min)

Additions	S77 medium	S77 + DEX medium
None	8.1	74.6
Dibutyl cyclic AMP	8.2	151.6
Dibutyl cyclic AMP + cycloheximide	7.9	87.2

Effect of inhibition of protein synthesis on reinduction. Cultures of HTC cells grown for 6 d in either S77 medium or S77 + dexamethasone, were each divided into three samples and resuspended at 500,000 cells  $\text{ml}^{-1}$ . Cycloheximide (0.1 mM, Sigma) was added to one sample from each culture; 30 min later 1 mM db cyclic AMP was added to each of these, and to a second sample. One sample from each original culture received nothing. Four hours after addition of the db cyclic AMP, TAT activity was determined in all samples. These results are representative of four similar experiments.

commonly ascribed to deletion or inactivation of genes during transformation or adaptation of cells to permanent growth in culture, but may also result from failure of the artificial milieu to support such processes. This demonstration of the restoration of a regulatory step by the addition of a hormone to the culture medium suggests that proper culture conditions may restore other apparently 'lost' functions in cultured hepatoma cells.

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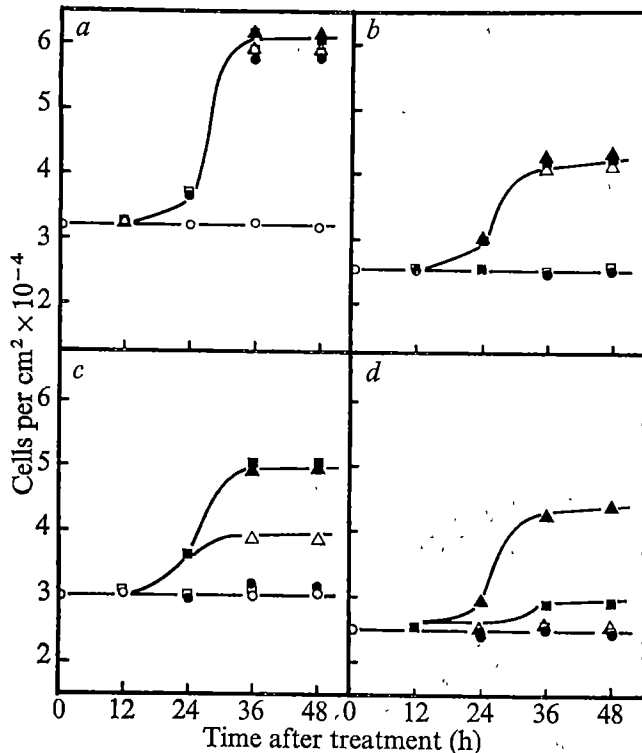
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## Role of serum in protease-induced stimulation of 3T3 cell division past the monolayer stage

TREATMENT of a confluent, quiescent 3T3 mouse embryo fibroblast cell monolayer with low concentrations of various proteases is sufficient to induce a new round of cell division<sup>1,2</sup>. It was suggested that modification of the 3T3 surface conformation from the non-agglutinable to the agglutinable state after protease treatment might have been responsible for the initiation of this round of cell division. More recently, however, Glynn *et al.*<sup>3</sup> demonstrated that modification of the 3T3 cell surface from the agglutinable to the non-agglutinable state was not sufficient in itself to induce a new round of cell division. In the light of this and other unpublished work from our laboratory I have re-evaluated the role of various culture conditions in the induction of 3T3 cell division after a brief treatment with Pronase. I found that induction of cell division after treatment of a confluent 3T3 monolayer with protease depends both qualitatively and quantitatively on the serum in the growth medium and that this requirement varies from cell line to cell line. Furthermore, my results suggest that the modification in surface architecture detected as enhanced agglutinability after brief proteolysis is not sufficient to induce a new round of cell division.

As Table 1 shows, four of the 3T3 cell lines tested showed remarkably different serum requirements for growth to the monolayer stage, which we have somewhat arbitrarily put at  $2.0 \times 10^4$  cells per  $\text{cm}^2$ . For example, the cell line designated 3T3<sub>3</sub> grows to the monolayer stage in Dulbecco's



**Fig. 1** Protease stimulation of different 3T3 cell lines in various concentrations of calf serum. The various 3T3 cell lines were plated at  $2.0 \times 10^3$  cells per  $\text{cm}^2$  and then grown to confluency in DME+10% calf serum. After 2 d at the monolayer stage the cells were washed once with sterile, phosphate-buffered 0.16 M NaCl (pH 7.2) (PBS). The cells were incubated for 5 min with 10  $\mu\text{g}$  of Pronase in 1.0 ml sterile PBS at room temperature. Cells were then washed with sterile PBS. DME plus the desired concentration of calf serum was then added back to the cells. Cells were counted every 12 h after stimulation. a, 3T3<sub>3</sub>; b, 3T3<sub>5</sub>; c, 3T3<sub>7</sub>; d, 3T3<sub>10</sub>. □, DME+3% calf serum; △, DME+5% calf serum; ■, DME+7% calf serum; ▲, DME+10% calf serum; ●, conditioned media; ○, untreated monolayer. "Conditioned" medium is defined in the legend to Table 1. The untreated monolayer refers to cells which, after growth to the monolayer, were not treated with Pronase or fresh calf serum and which serve as a base line from which the percentage stimulation is determined.

modified Eagle's medium (DME) plus as little as 3% calf serum while the 3T3<sub>10</sub> cell line requires DME plus 10% calf serum to reach the monolayer. Cell lines 3T3<sub>3</sub> and 3T3<sub>7</sub> have intermediate serum requirements, growing to  $2.0 \times 10^4$  cells per  $\text{cm}^2$  in DME plus 5% or 7% calf serum, respectively. Although two of these cell lines (3T3<sub>3</sub> and 3T3<sub>7</sub>) can grow to confluency in relatively low concentrations of calf serum, these lines do not significantly overgrow the monolayer when grown from low density in DME+10% calf serum.

I investigated the ability of DME plus various concentrations of calf serum to re-initiate cell division past the monolayer stage. As Table 2 shows, these cell lines vary considerably in terms of the concentration of calf serum necessary to stimulate a round of division past the monolayer. For example, 3T3<sub>3</sub> shows a 30% increase in cell number when DME+5% calf serum is added to the confluent cells whereas the other cell lines show no more than a 10% increase in cell number after a change of medium to DME+5% calf serum. In this same regard 3T3<sub>3</sub> cells show almost a 95% increase in cell number when DME+10% calf serum is added to the confluent cells, whereas 3T3<sub>10</sub> cells show only a 15% increase in cell number after a media change to DME+10% calf serum. 3T3<sub>3</sub> and 3T3<sub>7</sub> cells show responses to the various serum concentrations intermediate to 3T3<sub>3</sub> and 3T3<sub>10</sub>. None of the cell lines shows more than a 15% increase in cell number when DME plus that concentration of serum necessary for growth to

confluency is added to cells which have been grown to the monolayer stage in DME+10% calf serum.

To determine whether the differences in serum concentration required for subconfluent growth influenced the ability of the cells to overgrow the monolayer after protease treatment, each cell line was grown to confluency in DME+10% calf serum and then pulsed with Pronase as described in the legend to Fig. 1. As can be seen, there was significant induction of cell division after protease treatment only at a concentration of calf serum equal to or above that required for growth to the monolayer stage. After stimulation of each cell line in medium containing the minimal concentration of fresh calf serum sufficient to maintain cell division at a subconfluent density, a 65–75% increase in cell number was observed within 30 h of the initial stimulation. Only the 3T3<sub>3</sub> cell line showed a greater than 75% increase in cell number after treatment with Pronase.

To evaluate properly the effect of protease on the induction of cell division it is necessary to compare the stimulation of cell division observed before and after addition of fresh calf serum. Comparison of Fig. 1 and Table 2 shows that protease treatment lowered the concentration of fresh calf serum necessary for significant induction of cell division past the monolayer. For example, whereas 3T3<sub>3</sub>+DME containing 3% calf serum showed only a 10% induction of cell division (Table 2), 3T3<sub>3</sub> cells treated with protease before addition of DME+3% serum showed a 90% increase in cell number. Similar results have been obtained with the other three cell lines (compare Fig. 1 and Table 2).

**Fig. 2** Effect of ISI and Gibco calf serum on subconfluent cell growth and protease-stimulated overgrowth. 3T3<sub>10</sub> cells were plated at  $2.5 \times 10^3$  cells per  $\text{cm}^2$  in DME+10% ISI calf serum or 10% Gibco calf serum. Cell counts were taken daily during the next 6 d. Inset: 3T3<sub>10</sub> cells grown to confluency in DME+10% ISI calf serum or 10% Gibco calf serum were treated with 10  $\mu\text{g}$  of Pronase per ml PBS and then DME+10% ISI or 10% Gibco calf serum was added back to the cells at the monolayer. Cell counts were performed every 12 h for the next 72 h. ●, DME containing 10% Gibco calf serum; ■, DME containing 10% ISI calf serum; ○, Pronase 10  $\mu\text{g}$   $\text{ml}^{-1}$  + DME containing 10% Gibco calf serum; □, Pronase 10  $\mu\text{g}$   $\text{ml}^{-1}$  + DME containing 10% ISI calf serum. Addition of fresh 10% Gibco calf serum to cells at the monolayer without previous protease treatment produced an increase in cell number of approximately 15%. The addition of fresh 10% ISI serum to cells at the monolayer without previous protease treatment produced less than a 10% increase in cell number.

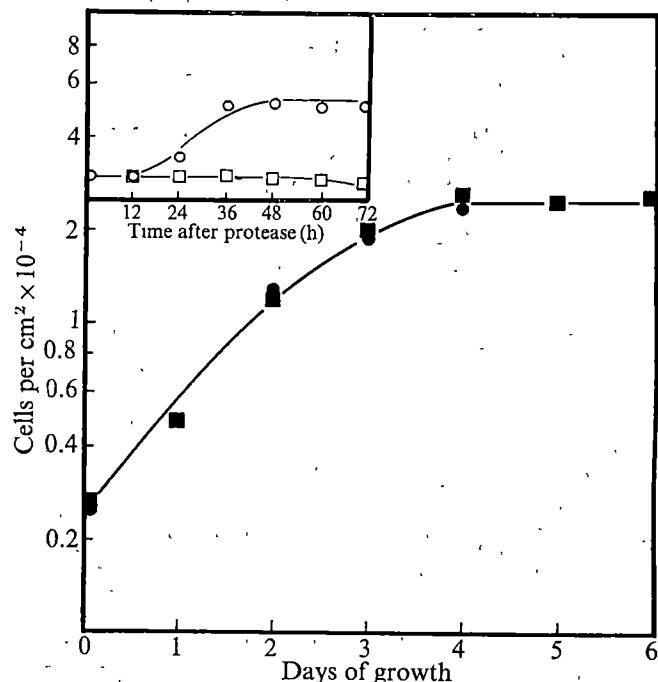


Table 1 Serum requirements of individual cell lines

Cell line	Final cell density cells per cm <sup>2</sup> × 10 <sup>-4</sup>				
	DME+3% calf serum	DME+5% calf serum	DME+7% calf serum	DME+10% calf serum	Conditioned medium
3T3 <sub>3</sub>	2.2	2.5	3.0	3.2	2.5
3T3 <sub>5</sub>	1.5	2.0	2.4	2.6	1.75
3T3 <sub>7</sub>	0.35	1.0	2.5	3.0	0.75
3T3 <sub>10</sub>	0.30	0.675	1.1	2.5	0.30

Saturation density was determined by plating cells at approximately  $2.0 \times 10^3$  per cm<sup>2</sup> in DME plus the given concentration of calf serum and counting cells daily for the next 7 d (ref. 2). A complete monolayer is formed with these cells at approximately  $2.0 \times 10^4$  cells per cm<sup>2</sup>. Cell size remained approximately the same for all cell lines tested. Cell lines originally derived from an NIH Swiss mouse embryo fibroblast (3T3) cell line were used throughout. The original line was obtained as clone 41 from Dr G. J. Todaro. Random stocks of this original clone had been stored in liquid nitrogen at different intervals during the past 5 yr. Four of these cell stocks were used. All cell lines were maintained at 37.5 °C in a moist incubator in which the CO<sub>2</sub> tension was held at 5%. Dulbecco's modified Eagle's medium (DME) plus 10% calf serum and 1% penicillin-streptomycin was used as growth medium for all stock cultures. The cells were demonstrated to be free of contamination with pleuro-pneumonia-like organisms (PPLO) at the time of the experiments, by both autoradiography<sup>6</sup> and mycoplasma broth media<sup>7</sup>. No cell line was maintained in culture for more than 20 passages. Frozen stocks were removed from liquid N<sub>2</sub> at intervals during the course of the experiments. "Conditioned" medium was produced by plating each cell line at  $2.0 \times 10^3$  cells per cm<sup>2</sup> in DME+10% calf serum and allowing the cells to grow in this medium for 5 d at which time the medium was removed and filtered. In this regard it must be noted that the term "conditioned" medium refers to medium taken from specific cell lines. Thus the "conditioned" medium which is added back to 3T3<sub>3</sub> cells is media which have been depleted by 3T3<sub>3</sub> cells while "conditioned" media which are added to 3T3<sub>10</sub> cells have been depleted by 3T3<sub>10</sub> cells. Since these cell lines have different serum requirements for growth it might be expected that the "conditioned" media produced by the two cell types might also vary.

Significantly, comparison of Table 2 and Fig. 1 demonstrates that 3T3<sub>3</sub> is the only cell line which, after growth to confluency, can be stimulated to divide in the presence of "conditioned" medium (see legend to Table 1 for definition of "conditioned" medium).

To determine whether the ability of serum to support growth past the monolayer varied with different lots of calf serum, Pronase stimulation of 3T3<sub>10</sub> cells was studied with three different sera. Figure 2 shows that subconfluent

It has been suggested that the surface modification detected as enhanced agglutinability with plant lectins, which results from mild protease treatment of a confluent monolayer, might be necessary for induction of cell division past the monolayer stage<sup>1</sup>.

The surface change detected as enhanced agglutinability is not sufficient for induction of cell division past the monolayer stage. Although 3T3<sub>10</sub> cells treated with 10 µg of Pronase per ml of sterile phosphate-buffered saline show

Table 2 Initiation of cell division past the monolayer by serum

Cell line	Final cell density per cm <sup>2</sup> × 10 <sup>-4</sup>	Cell density (per cm <sup>2</sup> × 10 <sup>-4</sup> ) after addition of DME at the indicated concentration of fresh calf serum to confluent cells					Conditioned medium
		3%	5%	7%	10%		
3T3 <sub>3</sub>	3.2	3.5	4.16	5.6	6.2		3.7
3T3 <sub>5</sub>	2.6	2.6	2.9	3.12	4.55		2.8
3T3 <sub>7</sub>	3.0	3.2	3.3	3.5	4.1		3.0
3T3 <sub>10</sub>	2.6	2.6	2.4	2.7	2.9		2.4

Each cell line was grown to confluency in DME+10% calf serum. The cells were left at confluency for 48 h and then the medium was changed to DME + the desired concentration of calf serum. Cell counts were performed every 12 h. In this table only the final cell density reached 48 h after the change of media is reported. "Conditioned" medium is defined in the legend to Table 1.

growth of the 3T3<sub>10</sub> cells is identical in calf serum obtained from Gibco (Lot No. C345417) and ISI (Lot No. 716C312). But as Fig. 2 shows, this lot of ISI sera did not support protease-induced cell division past the monolayer stage. This suggests that the serum components necessary for protease induction of cell division past the monolayer are either present in reduced amounts or missing completely from this lot of ISI sera. Calf serum obtained from Flow Laboratories was similar in its growth promoting characteristics to that of the Gibco calf serum used throughout these experiments.

enhanced agglutinability with wheat germ agglutinin and concanavalin A immediately after treatment (Table 3), these cells will complete a round of cell division only when exposed to DME plus fresh 10% calf serum (Fig. 1). Thus, as Glynn *et al.* suggested<sup>3</sup>, modification of the surface architecture from the non-agglutinable to the agglutinable state is not sufficient in itself to reinitiate the cell cycle in 3T3 cells at high density.

The work presented here suggests that the efficiency of protease-induced reinitiation of cell division varies from cell clone to cell clone, even within the 3T3 cell line. The

Table 3 Effect of protease on 3T3<sub>10</sub> cell growth and agglutination

Cell line	Final cell density (cells per cm <sup>2</sup> × 10 <sup>-4</sup> )		% Agglutination			
	Control	Plus Pronase	25 µg ml <sup>-1</sup> con A	25 µg ml <sup>-1</sup> WGA	25 µg ml <sup>-1</sup> con A	25 µg ml <sup>-1</sup> WGA
3T3 <sub>10</sub>	3.0	4.8	10%	10%	90%	95%

All agglutinations were performed as before<sup>3</sup>. DME+10% calf serum was added to the 3T3<sub>10</sub> cells after Pronase treatment. In the growth experiments "control" 3T3<sub>10</sub> cells were treated with DME+10% calf serum without previous Pronase treatment. With regards agglutination, control cells refer to cells before Pronase treatment. All protease treatments were performed with 10 µg Pronase per ml of sterile PBS for 5 min at 22 °C. Concanavalin A was prepared according to the techniques of Agrawal and Goldstein<sup>4</sup>, while wheat germ agglutinin (WGA) was prepared according to Nagata and Burger<sup>5</sup>.



relative efficiency of "protease-induced overgrowth" of the monolayer seems to correlate with the serum requirement which each cell clone expresses for sub-confluent growth. Thus in our work only those 3T3 cell lines which can grow to the monolayer in 3% calf serum seem able to respond to protease-induced reinitiation of the cell cycle without requiring the further addition of fresh serum to the growth medium.

In addition to this quantitative serum requirement there seems to be a qualitative requirement for a serum component(s) which is apparently not required for subconfluent growth but which is essential for growth past the monolayer. This conclusion stems from the observation that some calf sera which I tested, although able to support subconfluent growth, were unable to support growth of Pronase-treated cells past the monolayer.

Continuing work in our laboratory suggests that 3T3 cells may be peculiarly susceptible to induction of cell division after limited proteolysis. Attempts to induce other murine and rodent cell lines to reinitiate cell division after Pronase treatment have proved unsuccessful, even in media conditions which the work reported here suggests should be adequate for overgrowth.

Thus a working hypothesis is that limited protease digestion of the 3T3 cell membrane may act to increase the efficiency of utilisation of serum by a cell at the monolayer, thereby reinitiating cell division past the monolayer. This reinterpretation of earlier work is in substantial agreement with Holley's suggestion<sup>8</sup> that modification of the cell membrane may serve to increase the transport of serum nutrients or growth factors into the cell, and in this way lead to a reinitiation of the cell cycle. Reference 9 has a more extended discussion of the role of protease in cell growth.

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## Specificity and reversibility of interferon ganglioside interaction

RECENT studies have indicated that antiviral action of interferon is triggered by interaction with the cell membrane: Sepharose-bound mouse interferon (IF-Sepharose) retains its antiviral activity, and direct contact with the target cells is necessary to produce the antiviral effect<sup>1,2</sup>. Cells stimulated by double-stranded polyriboinosinic-polyribocytidylic acid to produce interferon are not protected from viral infection in the presence of interferon antiserum, indicating that interferon acts only after it has left the cells in which it is produced<sup>3</sup>. The same conclusion has been drawn from experiments with ouabain which inhibits the action, but not the production of primate interferon: cells stimulated to produce interferon fail to become antiviral in the presence of ouabain<sup>4</sup>.

We have shown previously that preincubation of mouse

L cells with *Phaseolus vulgaris* phytohaemagglutinin (PHA) blocks interferon action, suggesting the involvement of PHA-binding sites on the cell surface in cell membrane-interferon interaction<sup>5</sup>. We further demonstrated that Sepharose-bound mouse interferon is inhibited after preincubation with gangliosides, that soluble interferon binds to Sepharose-bound gangliosides, and that binding to gangliosides is inhibited by PHA<sup>6</sup>. Since gangliosides are cell membrane constituents whose carbohydrate portion faces the outside of the cell, we have investigated whether interferon-ganglioside interaction involves binding to the carbohydrate moiety of the gangliosides, thus supporting our hypothesis that interferon can interact with gangliosides on the outside of the cell membrane *in vivo*.

As Table 1 shows, when mouse IF-Sepharose was incubated with various glycolipids at comparable concentrations, pronounced inhibition of antiviral activity was observed with G<sub>M2</sub>, in accord with previous results<sup>6</sup>. Very little inhibition by G<sub>M3</sub> was seen in the conditions used, suggesting that the terminal *N*-acetyl-galactosaminy residue is an important constituent of the inhibitory molecule (see legend to Table 1). The same is true for terminal sialic acid as shown by the lack of inhibition obtained with ganglioTri-Cer. Both globoTri-Cer and globoTet-Cer (globoside) likewise were not inhibitory. They differ from G<sub>M2</sub> by the absence of sialic acid and by the fact that the galactosyl residue of lactosyl ceramide is substituted by an  $\alpha$ -1,4-linked galactosyl residue (globoTri-Cer) which in globoTet-Cer is further substituted by  $\beta$ -1,3-linked *N*-acetyl-galactosamine. GloboTri-Cer resulted in a slightly turbid solution in water, whereas ganglioTri-Cer yielded a turbid suspension, yet finely dispersed. Therefore in further experiments IF-Sepharose was preincubated with ethanolic solutions of the glycolipids. IF-Sepharose was first washed with absolute ethanol, which had little effect on its antiviral activity, then incubated with glycolipid solutions in ethanol followed by washing with water as described in the legend to Table 1. In these conditions only globoTet-Cer remained largely insoluble. The data corroborate those obtained with aqueous solutions, though G<sub>M3</sub> showed slight inhibition of antiviral activity. It seems that in ethanol, lower concentrations of G<sub>M2</sub> are required for complete inhibition, presumably because it forms micelles when dissolved in water. Since modification of the carbohydrate portion of G<sub>M2</sub> results in reduced inhibition or no inhibition, it seems that inhibitory ganglioside molecules bind through their carbohydrate portion to the interferon molecule and that optimal binding and inhibition require a lactosyl residue with galactose substituted by both a 2,3-linked sialic acid and a  $\beta$ -1,4-linked *N*-acetyl-galactosamine.

There is further support that ganglioside binding to interferon is not due to interaction of its lipid portion with hydrophobic sites on the interferon molecule. It was not possible to restore antiviral activity by washing IF-Sepharose, completely inhibited after preincubation with bovine brain gangliosides<sup>6</sup>, with 50% ethylene glycol. Such treatment reverses hydrophobic binding of human interferon to Sepharose columns containing covalently linked albumin<sup>12</sup>, or to CH-Sepharose<sup>13</sup>.

If inhibition of antiviral activity by gangliosides is due to specific interaction of interferon with carbohydrate constituents on the ganglioside molecule, it should be possible to reverse inhibition using saccharides resembling part or all of the carbohydrate structure of G<sub>M2</sub>. As Table 2 shows, when ganglioside-inhibited IF-Sepharose beads were washed with sialyl-lactose at pH 2, almost all antiviral activity reappeared. Washing with lactose or sialic acid alone had little effect. But when both sialic acid and lactose were present, antiviral activity was partially restored. Thus interferon seems to act like a lectin that is specific for at least the sialyl-lactose portion on the ganglioside molecule. At pH 7.0 and comparable concentrations neither sialyl-

**Table 1** Inhibition of antiviral activity of IF-Sepharese by glycolipids

IF-Sepharese preincubated with	Virus yield (% of control) after treatment of L cells with IF-Sepharese preincubated with glycolipids in:	
	Water	Ethanol
Nothing	0.7	2.5
G <sub>M2</sub>	51*	100
G <sub>M3</sub>	1	16
ganglioTri-Cer	1	5
globoTri-Cer	0.8	4.7
globoTet-Cer	1.2	5

IF-Sepharese prepared as before<sup>1,2</sup> was treated with glycolipids by incubating  $5 \times 10^4$  beads with 0.3 ml of a glycolipid solution in either water ( $3 \text{ mg ml}^{-1}$ ) or absolute ethanol ( $1 \text{ mg ml}^{-1}$ ). Beads preincubated with ethanolic solutions were washed first with absolute ethanol (twice, 2 ml). Incubation was for 75 min at  $37^\circ\text{C}$ . The beads were then washed twice with 2 ml water and suspended in 1 ml Eagle's minimal essential medium plus Hanks salts without serum (MEM). G<sub>M2</sub> was obtained from human brain<sup>7</sup>, G<sub>M3</sub> from bovine spleen<sup>7</sup>, ganglioTri-Cer from guinea pig erythrocytes<sup>8</sup>, globoTri-Cer from rabbit erythrocytes<sup>9</sup>, and globoTet-Cer from pig heart<sup>9</sup>. All glycolipids were essentially pure compounds according to thin layer chromatography and gas-liquid chromatography-mass spectrometry<sup>10,11</sup>. Suspensions of glycolipids not immediately soluble were heated to  $50^\circ\text{C}$  followed by two to four ultrasonic shocks of 2–3 s in a Heat Systems Sonifier (Model W185) at 50-W output. Mouse L cells were cultivated at  $37^\circ\text{C}$  in 35-mm plastic dishes ( $10^6$  cells per dish) in MEM, containing 10% calf serum. After removal of medium, cells were incubated for 6 h at  $37^\circ\text{C}$  with 1-ml suspensions of IF-Sepharese at a ratio of one bead per 20 cells. After removal of beads, cells were challenged with encephalomyocarditis virus (EMC) at a multiplicity of infection of 1. Virus yield was determined after 16 h of incubation at  $37^\circ\text{C}$  by counting plaque-forming units (PFU). The control virus yield per plate in the absence of interferon was  $6 \times 10^7$  PFU for the experiment with aqueous solutions and  $1.6 \times 10^8$  PFU for that with ethanolic solutions of glycolipids. We reported previously inhibition of mouse IF-Sepharese by a preparation of G<sub>M3</sub> containing both the acetyl and the glycolyl neuraminic acid derivatives<sup>6</sup>. Since that experiment was not carried out simultaneously with those testing the effects of the other individual gangliosides and since a different preparation of IF-Sepharese was used, the observed degree of inhibition by G<sub>M3</sub> in our earlier report is not directly comparable with that of the other gangliosides and to the results reported here. We found subsequently that different preparations of IF-Sepharese are inhibited to a different extent by the same quantity of a given ganglioside in the preincubation mixture. But in all IF-Sepharese preparations tested the relative order of effectiveness of the gangliosides as inhibitors remained the same:  $G_{M2} \geq G_{T1} > G_{M1} \geq G_{D1a} > G_{M3}$ . These variations might be due to different amounts of contaminating proteins or glycoproteins bound to the Sepharese particles, as the interferon preparations used for coupling to Sepharese were not purified extensively<sup>1,2</sup>. When the same IF-Sepharese preparation was used, the degree of inhibition observed by a given amount of a ganglioside was reproducible in different experiments. Gangliosides are designated according to Svennerholm<sup>7</sup>: G<sub>M3</sub>, 3-O-(N-acetyl) neuraminyl-4-O-β-galactosyl-O-glucosyl-ceramide; G<sub>M2</sub>, 4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl)neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G<sub>M1</sub>, 3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl) neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G<sub>D1a</sub>, 3-O-(N-acetyl) neuraminyl-3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl) neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G<sub>T1</sub>, 3-O-(N-acetyl) neuraminyl-3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (8-O-(N-acetyl) neuraminyl-3-O-(N-acetyl) neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide. Other abbreviations used are: globoTri-Cer, 4-O-α-galactosyl-4-O-β-galactosyl-O-glucosyl-ceramide; globoTet-Cer, 3-O-β-N-acetyl-galactosaminyl-4-O-α-galactosyl-4-O-β-galactosyl-O-glucosyl-ceramide; ganglioTri-Cer, 4-O-β-N-acetyl-galactosaminyl-4-O-β-galactosyl-O-glucosyl-ceramide; sialyl-lactose, 3-O-(N-acetyl)neuraminyl-4-O-β-galactosyl-O-glucose. All sugars are of the D configuration \*At  $2 \text{ mg ml}^{-1}$  in the preincubation mixture.

lactose nor the mixture of lactose and sialic acid restored antiviral activity of ganglioside-inhibited IF-Sepharese to any great extent, suggesting that the negative charge on the sialyl residues at neutral pH impedes replacement of the ganglioside molecules from interferon. It should be pointed out that in the determinations of antiviral activity only differences greater than twofold were considered significant.

We could not assess whether the complete tetrasac-

charide unit of G<sub>M2</sub> is superior to sialyl-lactose in displacing inhibitory gangliosides from IF-Sepharese, since this tetrasaccharide was not available. In displacement experiments in which suboptimal concentrations of sialyl-lactose were used during washing of ganglioside-inhibited IF-Sepharese beads in otherwise identical conditions, N-acetyl-galactosamine did not enhance the effect of sialyl-lactose. Thus there was no difference in the virus yield after treatment of L cells with ganglioside-inhibited IF-Sepharese subsequently washed at pH 2.0 or pH 7.0 with 0.02 M sialyl-lactose, compared with experiments in which a solution containing 0.02 M sialyl-lactose plus 0.5 M N-acetyl-galactosamine was used for washing. Similarly, when 0.5 M N-acetyl-galactosamine was included with 0.1 M lactose and 0.1 M sialic acid in the solution used to remove bound gangliosides from IF-Sepharese, the effect observed with the mixture of sialic acid and lactose alone was not enhanced.

Since most glycoproteins contain terminal sialic acid often bound to penultimate galactosyl units, we surveyed some of these glycoproteins for possible inhibitory action on the antiviral activity of interferon. In these experiments  $5 \times 10^4$  IF-Sepharese beads were preincubated with 0.3 ml glycoprotein solution in water for 1 h at  $37^\circ\text{C}$  followed by washing with phosphate-buffered saline (PBS) (twice 2 ml). None of the following materials ( $20 \text{ mg ml}^{-1}$ ) had any effect on antiviral activity: Fetuin (Sigma), bovine submaxillary mucin (Sigma), rabbit α- and γ-globulins (Nutritional Biochemicals), human chorionic gonadotropin (Ayerst). Thus inhibition of IF-Sepharese not only requires the presence of sialyl or sialyl-galactosyl residues on a glycoconjugate, but seems to be more specific.

The fact that shortening of the saccharide portion of G<sub>M2</sub> can partially or completely prevent inhibition of interferon, supports our conclusion that interferon interacts with the carbohydrate moiety of this molecule. Substitution of the G<sub>M2</sub> tetrasaccharide unit by β-1,3-linked galactose as in G<sub>M1</sub> and higher gangliosides, has little influence on inhibitory potency<sup>6</sup>. Reversal of ganglioside inhibition by sialyl-lactose indicates that at least part of the binding site on the inter-

**Table 2** Effect of saccharide solutions on ganglioside-inhibited IF-Sepharese

Ganglioside-pretreated IF-Sepharese washed with:	Virus yield (% of control) after incubation of L cells with ganglioside-pretreated IF-Sepharese washed subsequently at	
	pH 2	pH 7
Buffer	54	72
0.1 M sialic acid	26	25
0.1 M lactose	35	50
0.1 M lactose plus 0.1 M sialic acid	8	30
0.7 M lactose plus 0.7 M sialic acid	11	ND
0.1 M sialyl-lactose	1.8	26
IF-Sepharese not preincubated with gangliosides	0.3	0.3

IF-Sepharese ( $5 \times 10^4$  beads) was incubated with 0.3 ml of a solution of mixed gangliosides from bovine brain (Sigma, type III,  $2 \text{ mg ml}^{-1}$ ) for 75 min at  $37^\circ\text{C}$ . The beads were washed twice with 2 ml of water to remove unbound gangliosides. They were then suspended in 0.4 ml of the indicated saccharide solutions either in 0.2 M glycine-HCl buffer at pH 2.0 or in 0.3 M sodium phosphate buffer, pH 7.0. The pH was readjusted where necessary. After incubation at  $37^\circ\text{C}$  for 30 min with occasional stirring the supernatant solution was removed, and the beads were washed twice more with 2 ml of water and finally suspended in 1 ml of MEM. The major constituents present in the mixed ganglioside preparation used were G<sub>M1</sub> and G<sub>D1a</sub>, as revealed by thin-layer chromatography in chloroform-methanol-2.5 N ammonia (60:40:9; v/v). The sialyl-lactose used was from bovine colostrum (Sigma) and contained approximately 85% of the 2,3-linked and approximately 15% of the 2,6-linked N-acetyl-neuraminyl derivatives. Antiviral activity was determined as described in the legend to Table 1. The control virus yield per plate was  $10^8$  PFU. ND, not determined.

feron molecule is directed towards this trisaccharide unit. The fact that neither sialic acid nor lactose alone can reverse inhibition of IF-Sepharose by gangliosides, whereas a mixture of both is effective to some extent, supports our conclusion that the binding site for interferon includes both sugar residues. This is analogous to the observed inhibition of PHA-induced red cell agglutination by an erythrocyte glycopeptide that contains the PHA receptor site: although removal of galactose from this glycopeptide abolishes its agglutination-inhibitory activity, neither galactose alone nor  $\beta$ -galactosyl-*N*-acetyl-glucosaminyl-containing oligosaccharides are inhibitors of PHA-induced haemagglutination. Thus, although galactosyl residues are essential for PHA binding, the complete binding site seems to include further saccharide units<sup>14</sup>.

The relatively poor inhibition observed with  $G_{M3}$  suggests that optimal binding of interferon to gangliosides includes interaction with the  $\beta$ -1,4-linked *N*-acetyl-galactosaminyl residue as well. This hypothesis could be substantiated further by comparing the relative efficiencies of removing inhibitory gangliosides from IF-Sepharose using either sialyl-lactose or the tetrasaccharide derived from  $G_{M2}$ . Thus with the latter, lower concentrations should be sufficient to restore completely antiviral activity of ganglioside-inhibited IF-Sepharose. It is not obvious why sialyl-lactose reverses ganglioside inhibition at pH 2.0 but not at pH 7.0. Possibly the negative charges on the ganglioside molecules at pH 7.0 repel the negatively charged sialyl-lactose residues sufficiently to prevent successful competition of the trisaccharide with the bound gangliosides. This phenomenon would not occur at pH 2.0, where sialyl residues are uncharged.

Cells transformed by RNA and DNA viruses often have altered ganglioside patterns<sup>15,16</sup>. For example, transformation of mouse cells by murine sarcoma virus (Moloney strain) yields decreased levels of  $G_{M2}$  and higher gangliosides in the cell membrane<sup>17</sup>. Virus transformation often results in decreased sensitivity to the antiviral effect of interferon<sup>18</sup>. Involvement of higher gangliosides in the cell membrane receptor sites for interferon could explain the poor antiviral response of some transformed cells. Preincubation of SV40-transformed mouse cells with gangliosides increases their sensitivity towards the antiviral effect of interferon, in accord with such a hypothesis<sup>19</sup>.

Binding of interferon to Sepharose-bound gangliosides<sup>9</sup> and elution with the appropriate saccharide solutions suggests a way to purify interferon by affinity chromatography. Such studies are in progress.

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## Thrombin-sensitive surface protein of cultured chick embryo cells

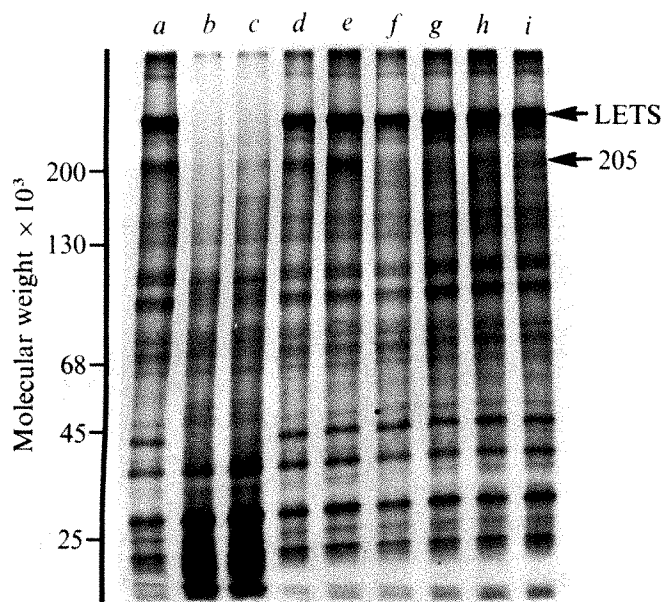
THROMBIN, a serine protease with great specificity, is a potent mitogen for resting chick embryo fibroblasts in culture<sup>1-3</sup>. So far only a limited number of peptide bonds in a few native proteins have been found to be sensitive to proteolysis by thrombin, namely four Arg-Gly bonds in fibrinogen<sup>4</sup> and a few arginyl and lysyl bonds in actin<sup>5</sup>. It is likely therefore that the number of fibroblast surface proteins susceptible to this protease will also be limited, whereas with trypsin, many surface proteins are attacked. Thus thrombin may be an excellent probe for studies of the role of changes in cell surface composition during mitogenesis or cell-cell interaction. A large, external transformation sensitive (LETS) protein<sup>6-10</sup>, postulated<sup>10,11</sup> to be involved in cellular growth control, is insensitive to thrombin<sup>2</sup>. This observation led to the conclusion that the removal of LETS is not a necessary condition for cell growth when a protease is used to stimulate growth.

Using a lactoperoxidase-catalysed <sup>125</sup>I-iodine labelling technique, we were unable previously to detect decisively any thrombin-sensitive chick fibroblast surface protein. Yet since thrombin has a potent effect on chick embryo fibroblasts, it seemed likely that some surface protein is sensitive to its proteolytic activity. Identification of such a protein may be important not only for the understanding of cell surface composition, but also for the elucidation of the mechanism of protease-induced mitogenesis.

We have found that when <sup>125</sup>I-iodine is substituted for <sup>125</sup>I-iodine in lactoperoxidase-catalysed iodination, the method greatly increases the number of surface proteins detectable by gel electrophoresis and autoradiography. This observation led to the finding reported here that there is a thrombin-sensitive protein with an apparent molecular weight of 205,000 on the surface of chick cells.

Until recently, the usual procedure for iodination of proteins has involved <sup>125</sup>I-iodide, mainly because of its more convenient long half life (60 d). When autoradiograms of slab gels of solubilised, iodinated cells are made, however, <sup>125</sup>I-iodide yields poor resolution because of its high energy gamma emission. A  $\beta$ -emitting iodine isotope would overcome this difficulty, since  $\beta$  radiation is captured more efficiently by the photographic emulsion than is  $\gamma$  radiation; the scattering effect is greatly reduced and resolution much improved. The combination of the use of <sup>131</sup>I-iodide instead of <sup>125</sup>I-iodide and a gradient polyacrylamide gel is recommended. The improvement in analytical power offsets the inconvenience of a shorter half life (8 d).

As Fig. 1 shows, use of <sup>131</sup>I-iodide and electrophoresis on sodium dodecyl sulphate (SDS)-polyacrylamide gradient slab gels makes possible detection of a large number



**Fig. 1** Autoradiograms of SDS-polyacrylamide gradient gels of proteins labelled by lactoperoxidase-catalysed iodination of chick fibroblasts after the following treatment at 37 °C in Dulbecco's modified Eagle's medium (DEM) kept in a 10% CO<sub>2</sub> incubator. *a*, Untreated control, resting secondary chick cells prepared by seeding  $4 \times 10^5$  cells per 35-mm dish in DEM+0.5% calf serum (Grand Island Biological) for 3 d; *b*, trypsin at  $0.2 \mu\text{g ml}^{-1}$  for 4 h; *c*, trypsin at  $0.1 \mu\text{g ml}^{-1}$  for 4 h; *d*, insulin at  $5 \mu\text{g ml}^{-1}$  for 4 h; *e*, insulin at  $1 \mu\text{g ml}^{-1}$  for 4 h; *f*, thrombin at  $50 \mu\text{g ml}^{-1}$  for 30 min; *g*, thrombin at  $20 \mu\text{g ml}^{-1}$  for 4 h; *h*, thrombin at  $2 \mu\text{g ml}^{-1}$  for 24 h; *i*, thrombin at  $1 \mu\text{g ml}^{-1}$  for 2 h. Washed cells were iodinated in 1 ml of Dulbecco's phosphate-buffered saline containing 0.9 mM CaCl<sub>2</sub> and 0.9 mM MgCl<sub>2</sub> with addition of 20  $\mu\text{g}$  of lactoperoxidase (Worthington) further purified by chromatography on a Sephadex G-100 column, 50  $\mu\text{Ci}$  of carrier-free Na<sup>125</sup>I (New England Nuclear) in 0.1 N NaOH, and 2.4 nmol of NaI. The reaction was initiated by addition of 5  $\mu\text{mol}$  of glucose and 0.015 U of glucose oxidase (Sigma). Reactions were terminated after 15 min by five washes of ice-cold Ca<sup>2+</sup>- and Mg<sup>2+</sup>-free phosphate-buffered saline solution. The cells were then dissolved in electrophoresis sample buffer containing 2% SDS, 1% mercaptoethanol and 2 mM phenylmethane sulphonyl fluoride. Slab gel electrophoresis was carried out as described previously<sup>2</sup> except that the acrylamide gradient was 6–15%. The radioactivity loaded on a slab gel was 20,000 c.p.m. for each sample. The slab gel was dried and subjected to autoradiography on Kodak RP Royal X-Omat X-ray film for 3–24 h. The standards used for molecular weight determination are myosin,  $\beta$ -galactosidase, bovine serum albumin, actin and chymotrypsinogen.

of iodlatable proteins, many more than in previous results<sup>2-4,7-10</sup>. Most of the bands in the autoradiogram are sharp and well defined compared with other reports where <sup>125</sup>Iodide was used. We find that one band (indicated by an arrow) is sensitive to thrombin. Column *f* shows that after treating the cells for 30 min with a high concentration of thrombin ( $50 \mu\text{g ml}^{-1}$ ) this protein can no longer be detected when compared with the external proteins from a control culture of resting chick cells (column *a*).

The experimental conditions for columns *g*, *h* and *i*, respectively, are: resting chick cells treated with thrombin at  $20 \mu\text{g ml}^{-1}$  for 4 h,  $2 \mu\text{g ml}^{-1}$  for 24 h and  $1 \mu\text{g ml}^{-1}$  for 2 h. A significant observation has been that in all these conditions one iodlatable protein of molecular weight 205,000 was consistently sensitive to thrombin. Figure 2 shows microdensitometer scans of two of the gel strips (*a* and *g*) of Fig. 1. These scans confirm quantitatively the absence of only one iodlatable cell surface protein after treatment of chick embryo fibroblasts with thrombin. This thrombin-sensitive protein, as expected, is also sensitive to trypsin.

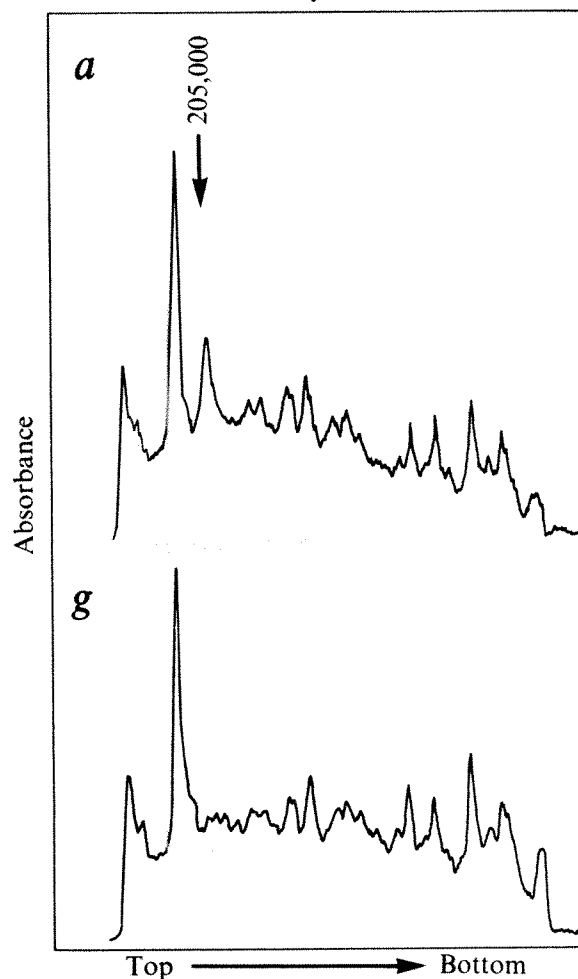
As in the previous report where <sup>125</sup>Iodide was used, we find that LETS protein is not removed by thrombin treat-

ment<sup>2</sup>. LETS protein stands out even more clearly as the most prominent cell surface protein in cases where <sup>131</sup>Iodine was used in place of <sup>125</sup>Iodide. As columns *b* and *c* show, both LETS and the 205,000 protein were removed by trypsin. On the other hand, insulin at  $1 \mu\text{g ml}^{-1}$  or  $5 \mu\text{g ml}^{-1}$  for 4 h does not remove thrombin-sensitive protein (columns *d* and *e*).

When serum proteins were labelled with <sup>131</sup>Iodine by lactoperoxidase-catalysed reaction, no iodlatable protein comigrating with the thrombin-sensitive protein was detected. Furthermore, the availability of this thrombin-sensitive protein for iodination on the cell surface is not serum dependent. Chick embryo cells passaged in serum-free medium (but supplemented with insulin at  $5 \mu\text{g ml}^{-1}$ ) have the same amount of thrombin-sensitive protein as that of cells grown in 10% serum. Finally, sparse, exponentially growing chick cells incubated in 10% serum have only a very small amount of thrombin-sensitive protein. Therefore, it is unlikely that this protein is a component of serum. Since it has been established<sup>6-10</sup> that lactoperoxidase-catalysed iodination labels only proteins on the surface of cells, this thrombin-sensitive protein is probably an external cell-surface protein. The possibility that this thrombin-sensitive protein is released by dead cells in culture is ruled out because we used only cultures of secondary chick cells, which have no detectable. Trypan blue-positive cells both before and after iodination.

The role of the 205,000 protein is unknown. It is possible

**Fig. 2** Densitometric scans of gel strips *a* and *g* of Fig. 1. Two of the autoradiograms shown in Fig. 1 (*a* and *g*) were scanned by a Canalco Model G 11 densitometer with a digital integrator (Autolab 6300) attachment. Since the thrombin-treated cultures (*f*–*i*) gave essentially the same scanning results only *g* was shown in this figure. The scan of gel strip *a* represents the untreated control. Thrombin-sensitive proteins are indicated by an arrow.





that thrombin attacks the 205,000 protein proteolytically and that this event triggers renewed cell proliferation. Alternatively, the effect of thrombin on the resting cell might be stimulation of cell proliferation by some unknown mechanism concerned with altered cell-cell interaction in the absence of the external 205,000 protein. These two possibilities are being investigated.

Cell-surface proteins (including membrane proteins and surface coat proteins) are undoubtedly important in many complex biological phenomena. Progress in these studies has been slow because the role of a surface protein is always difficult to establish. An ideal way for the assignment of biological function to a surface component is the use of cell mutants lacking a specific protein(s). Alternatively, if a protease probe is available that can specifically alter one surface protein, the assignment of its function may be achieved by comparison with a control culture in which a protease has not been included. Use of highly specific proteases such as those involved in blood coagulation, for example, thrombin, factor X<sub>a</sub> and IX<sub>a</sub>, may prove to be fruitful for such work.

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## New structural characteristic of the large glycopeptides from transformed cells

IN comparative gel filtration of glycopeptides obtained from the surface of normal and transformed cells, an increased concentration of fucosyl glycopeptides of apparently large molecular weight was observed for cells transformed by viral, chemical and spontaneous methods<sup>1-7</sup>. This is considered to be an expression of transformed phenotype, since in cells transformed by a temperature-sensitive (*ts*) mutant of Rous sarcoma virus, the heavy glycopeptides increased only at the permissive temperature. Based on the results of sialidase treatment, it was suggested that the presence of extra sialic acid is responsible for the higher molecular weight nature of the glycopeptides from transformed cells<sup>4,6</sup>.

In this communication, however, we present evidence that the differences are not that simple, but are more

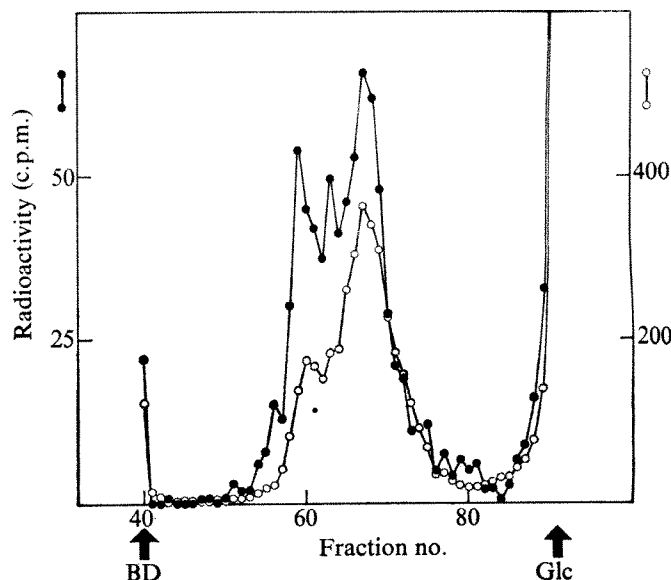
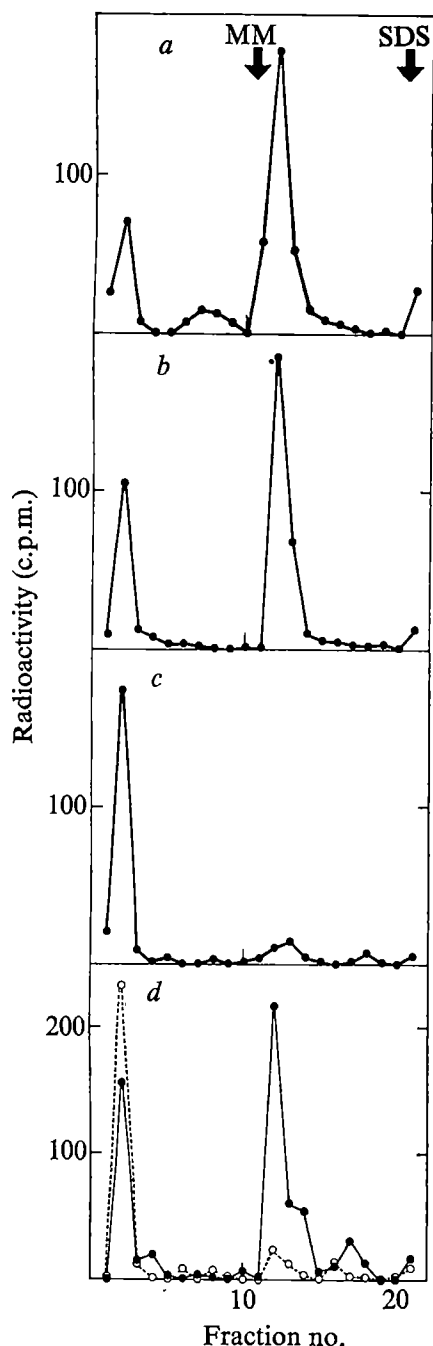


Fig. 1 Sephadex G-50 column chromatography of fucose-labelled glycopeptides from trypsinates of normal and polyoma transformed cells. BHK<sub>21-11</sub> cells and the cells transformed by polyoma virus were grown at 37 °C in plastic Petri dishes (growth area 78 cm<sup>2</sup>) containing 10 ml of Eagle's MEM supplemented with 10% foetal calf serum and antibiotics in a humidified CO<sub>2</sub> incubator. The inoculum was 1 × 10<sup>6</sup> cells per dish. Three days after plating, 30 µCi of <sup>3</sup>H-fucose (4.4 Ci mmol<sup>-1</sup>) or 3 µCi of <sup>14</sup>C-fucose (50.8 mCi mmol<sup>-1</sup>) was added to each dish. After 24 h, the cultures were washed twice with Earle's solution, and treated with 5 ml of 0.1% trypsin (Difco) in Earle's solution lacking CaCl<sub>2</sub> and MgSO<sub>4</sub> and containing 0.016% EDTA for 5 min at room temperature. Then the medium was carefully decanted and centrifuged at 500g for 5 min to remove any loose cells. The supernatant (termed as 'trypsinates') was digested with Pronase for 3 d adding 1 mg ml<sup>-1</sup> of Pronase every day. Then, Pronase was inactivated by heating at 100 °C for 5 min, and the digests were applied to a column of Sephadex G-50, fine (1.0 × 125 cm). The column was equilibrated and eluted with 0.1 M pyridine-acetate buffer, pH 5.5. Fractions (1 ml) were collected. Aliquots (0.3 ml) were removed for counting, and the rest of the material was pooled as the large glycopeptides (fraction 56 to 62) and the small glycopeptides (fraction 64 to 74), lyophilised and stored. The figure shows a typical double labelling experiment in which the glycopeptides from BHK cells (○) were labelled with <sup>14</sup>C and those from polyoma-transformed BHK cells (●) were labelled with <sup>3</sup>H. The standard glycopeptides, (Man)<sub>5</sub>(GlcNAc)<sub>2</sub>N-(<sup>14</sup>C)acetyl-Asn (molecular weight 1,393), <sup>14</sup>C-acetylated bovine IgG glycopeptides (molecular weight ~2,000) and <sup>14</sup>C-acetylated unit B glycopeptides of thyroglobulin (molecular weight ~3,000) were eluted in fractions 77-78, 72 and 66, respectively. Molecular weight of the large and the small glycopeptides was estimated by the method of Andrews<sup>18</sup>.

extensive, possibly including both galactose and N-acetylglucosamine.

The fucose-labelled large glycopeptides were prepared from the trypsinates of polyoma-transformed BHK cells, and the small glycopeptides from the trypsinates of the normal and the polyoma-transformed cells (Fig. 1). In agreement with the work of Warren *et al.*<sup>3,4</sup>, the large glycopeptides were more enriched in transformed cells. On paper electrophoresis at pH 5.4, the large glycopeptides migrated faster to the positive pole than the small glycopeptides. Neuraminidase treatment of these glycopeptides converted most of them into neutral glycopeptides, indicating that the negative charge of these glycopeptides arose from sialic acid. We could also, therefore, confirm that the large glycopeptides have more sialic acids than the small glycopeptides.

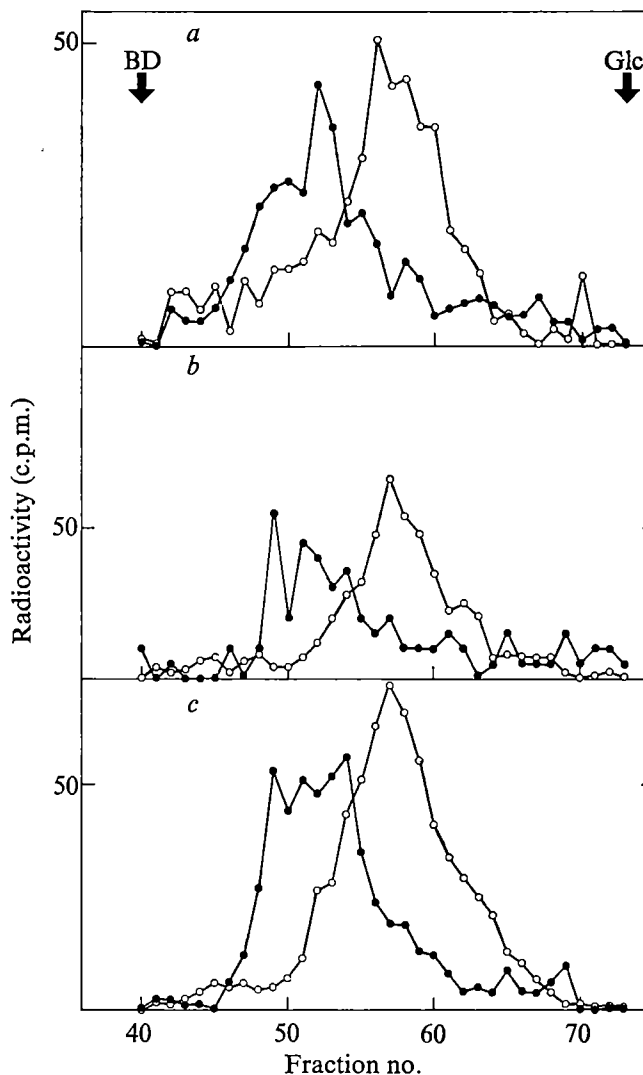
These glycopeptides were studied comparatively by affinity column chromatography on concanavalin A (con A)-Sephacrose<sup>8</sup>. About 70% of the small glycopeptides from both normal and polyoma-transformed cells were bound to the column. Most of the bound glycopeptides



**Fig. 2** Affinity column chromatography of glycopeptides on con A-Sepharose. *a*, The small glycopeptides from BHK cells; *b*, the small glycopeptides from polyoma-transformed BHK cells; *c*, the large glycopeptides from polyoma-transformed BHK cells, and *d*, the large glycopeptides from polyoma-transformed BHK cells treated with neuraminidase alone (.....) or with neuraminidase,  $\beta$ -galactosidase and  $\beta$ -N-acetylglucosaminidase (—). Glycopeptides and their derivatives were applied to a column of con A-Sepharose (Pharmacia, Sweden) equilibrated with 0.01 M Tris-HCl buffer, pH 7.5 containing 0.1 M NaCl. The column was washed with 10 ml of the above buffer, and then eluted with 10 ml of the same buffer containing 0.1 M methyl  $\alpha$ -mannoside. Fractions (1 ml) were collected. Then con A-Sepharose was extracted with 5 ml of 1% SDS at 100 °C for 2 min. Glycopeptides in 0.02 ml of 0.1 M phosphate buffer, pH 6.0 were mixed with 0.01 ml of neuraminidase from *Clostridium perfringens* (0.01 units, Sigma) and were incubated at 37 °C for 2 h. The neuraminidase-treated glycopeptides were heated at 100 °C for 5 min and adjusted to pH 4.0 by the addition of 0.01 ml of 0.15 M citrate-phosphate buffer, pH 3.0. Then, 0.04 ml each of  $\beta$ -galactosidase<sup>17</sup> (0.64 U) and  $\beta$ -N-acetylglucosaminidase<sup>18</sup> (3.7 U) from jack bean meal were added and the mixture was incubated at 37 °C for 16 h with a small amount of toluene.

were eluted by 0.1 M methyl  $\alpha$ -mannoside, and a small amount of tightly bound glycopeptides by 1% sodium dodecyl sulphate (SDS) (Fig. 2 *a*, *b*). In contrast, we found that only 20% of the large glycopeptides from polyoma-transformed cells were bound to the column (Fig. 2*c*). This behaviour of the large glycopeptides did not change, even after neuraminidase digestion (Fig. 2*d*, dotted line), indicating that the enrichment of sialic acid is not responsible. A new structural difference between the large and the small glycopeptides was therefore shown.

We then compared core structures of the large glyco-



**Fig. 3** Sephadex G-25 column chromatography of glycopeptides treated with glycosidases. *a*, The large glycopeptides from polyoma-transformed BHK cells; *b*, the small glycopeptides from polyoma-transformed BHK cells; *c*, the small glycopeptides from normal BHK cells. Chromatography was performed on a column of Sephadex G-25, fine (1  $\times$  125 cm) equilibrated and eluted with 0.01 M Tris-HCl buffer, pH 7.5 containing 0.1 M NaCl. Fractions of 1 ml were collected. The standard substances, (Man)<sub>6</sub>(GlcNAc)<sub>2</sub>N-(<sup>14</sup>C)-acetyl-Asn (molecular weight 1,393) and GlcNAc-N-(<sup>14</sup>C)-acetyl-Asn (molecular weight 379) were eluted in fractions 47–48 and 60–61, respectively. ●, Glycopeptides treated with neuraminidase,  $\beta$ -galactosidase and  $\beta$ -N-acetylglucosaminidase. The condition of exoglycosidase digestion was the same as described in Fig. 2. ○, Glycopeptides treated with endo- $\beta$ -N-acetylglucosaminidase D in the presence of neuraminidase,  $\beta$ -galactosidase and  $\beta$ -N-acetylglucosaminidase. The glycopeptides were digested with the mixture of 10.4 milliunits of endo- $\beta$ -N-acetylglucosaminidase D (ref. 9), 5.8 mU of neuraminidase<sup>19</sup>, 14.2 mU  $\beta$ -galactosidase<sup>19</sup> and 46 mU  $\beta$ -N-acetylglucosaminidase<sup>20</sup>, all from *Diplococcus pneumoniae* in 0.06 ml of 0.05 M citrate-phosphate buffer, pH 6.6 at 37 °C for 16 h. The products of the endoglycosidase digestion were concluded to be glycopeptide fragments and not oligosaccharides, since they migrated identically with the glycopeptide fragments from IgG glycopeptides on paper chromatography in butanol-acetic acid–1 N NH<sub>4</sub>OH (2:3:1).

peptides and the small glycopeptides. The cores were prepared by enzymatic removal of sialic acid, galactose and *N*-acetylglucosamine. We found that 70% of the cores both from the large glycopeptides of transformed cells (Fig. 2d, solid line) and from the small glycopeptides of normal and transformed cells (data not shown) were bound to the column of con A-Sepharose. Molecular weights of the cores were estimated to be ~1,100 in all the cases (Fig. 3). Further similarity of core structures was indicated by digestion with endo- $\beta$ -*N*-acetylglucosaminidase D (ref. 9). Most of the large and the small glycopeptides were hydrolysed by endo- $\beta$ -*N*-acetylglucosaminidase D in the presence of neuraminidase,  $\beta$ -galactosidase and  $\beta$ -*N*-acetylglucosaminidase, yielding glycopeptide fragments of molecular weight ~450 (Fig. 3). Therefore, in both the large and the small glycopeptides, fucose residue was located in the vicinity of protein-carbohydrate linkage region as described previously in human diploid cells<sup>10</sup>. In addition to the linkage region, clusters of mannosyl residues must be present in the cores of most of the glycopeptides, since both susceptibility to endo- $\beta$ -*N*-acetylglucosaminidase D (ref. 11) and binding to con A-Sepharose<sup>8</sup> required their presence. The number of the mannosyl residues in the glycopeptides were estimated to be ~3 in all the cases, since the difference of molecular weights between the core and the glycopeptide fragments was ~650, and the value roughly corresponded to the molecular weight of Man<sub>3</sub>GlcNAc, the principal oligosaccharide released from cell-surface glycopeptides by endo- $\beta$ -*N*-acetylglucosaminidase D (ref. 12). Therefore, cores of the large and the small glycopeptides were very similar to each other and to those of non-membrane glycopeptides such as IgG glycopeptides<sup>13,14</sup> (Fig. 4).

Summarising the results, the large glycopeptides and the small glycopeptides were different in their behaviour towards con A-Sepharose in spite of the close identity of the core structures. We have previously indicated that the presence of two  $\alpha$ -mannosyl residues capable of interacting with con A is required for a glycopeptide to bind to the column<sup>8</sup>. The interacting  $\alpha$ -mannosyl residues are those with free hydroxyl groups on C3, C4 and C6 (ref. 15). Thus, the large glycopeptides may have more branches composed of sialic acids, galactose and *N*-acetylglucosamine (Fig. 4). These additional branches will take the place of the hydroxyl groups of  $\alpha$ -mannosyl residues, resulting in the reduction of the number of  $\alpha$ -mannosyl residues interacting with con A.

The above conjecture is also supported by molecular weight values of the two glycopeptides. Calibrating the Sephadex G-50 column with standard glycopeptides, we could estimate approximate molecular weights of the large and the small glycopeptides to be 4,000 and 2,500, respectively (Fig. 1). Thus, the molecular weight difference of the large and the small glycopeptides was 1,500, which might be too large to be explained by the addition of sialic acid itself, but can be easily explained by the addition of whole chains of sialyl-galactosyl-*N*-acetylglucosaminyl

branches (Fig. 4). If the above inference is correct, the key step leading to the large glycopeptides is not sialylation<sup>4</sup>, but the initial formation of additional branches, possibly *N*-acetylglucosaminylation.

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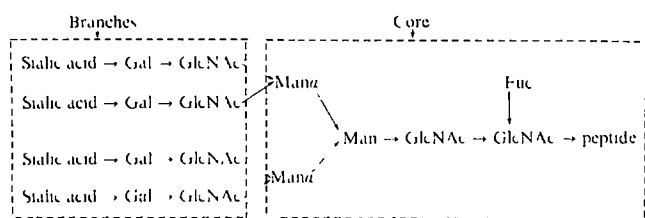
## Influenza A antigens on human lymphocytes *in vitro* and probably *in vivo*

INFLUENZA A viruses cause respiratory tract infections in man, associated with generalised symptoms of fever, headache and myalgia. Virus replication seems to be limited to the respiratory tract epithelium<sup>1</sup>, and attempts to demonstrate a viraemia have been largely unsuccessful<sup>2</sup>, although it is not unknown<sup>3</sup>. The mechanisms producing the generalised symptoms are not yet understood. In this paper we demonstrate the probable presence of viral antigens on peripheral blood lymphocytes from patients during peak infection with influenza. This could have important implications both for the pathogenesis of influenza and for early diagnosis.

The technique we used was the mixed antiglobulin rosette-forming reaction<sup>4</sup> (MAR), which involved two stages of lymphocyte sensitisation. The lymphocytes to be tested for adsorbed influenza antigens were first treated for 45 min at 4 °C with rabbit anti-influenza serum (R9 or R18; see footnote to Table 1), or with normal rabbit serum (NRS) or diluent as controls. All rabbit sera had been heat inactivated and absorbed  $\times 3$  with human erythrocytes and  $\times 3$  with human formalised leukaemic spleen cells to remove anti-species antibodies. The lymphocytes were washed twice after the first-stage sensitisation, and then exposed to a sheep (207, bleed 5) anti-rabbit globulin serum (45 min, 4 °C). They were washed twice more and centrifuged (350g, 2 min, 4 °C) for rosette formation with equal volumes (20-25  $\mu$ l) of 0.8% (v/v) sheep erythrocytes coated with immunoglobulin from a rabbit anti-sheep erythrocyte serum (R8104/II, dilution 1:10,000). The pelleted cells were gently resuspended and mixed with toluidine blue stain for microscopic examination.

Lymphocytes for examination were separated from

**Fig. 4** A structural model of the majority of the large glycopeptides from transformed cells. The number of branches was estimated to be ~4, since the molecular weight difference of the intact glycopeptides and the core was ~2,900. In the small glycopeptides, the core structure was probably identical, but the number of branches was considered to be smaller



**Table 1** Percentages of lymphocytes from six normal asymptomatic humans reacting in the MAR

Lymphocytes (formaldehyde treated) $2.5 \times 10^6 \text{ ml}^{-1}$	Serum HI titre	Serum used in first stage of sensitisation*						PBS/BSA controls
		R9 anti-MRC11 1:25	1:100	R18 anti-MRC2 1:25	1:100	NRS 1:25	1:100	
J.H.	10	2.5	2	3.5	3	4	3.5	2
Z.M.	10	5	3.5	2	0	1.5	2	2
K.A.	10	4	4	4	5	3	4	2
D.F.	10	3.5	4.5	5	4.5	2	2	0
M.F.	20	2	2	7	3	2	1.5	0
E.M.	10	3	2	6	5	5	4.5	2

\* Rabbit anti-influenza sera R9 raised against MRC11 recombinant strain, and R18 raised against MRC2 strain, both virus strains closely related antigenically to current strains with surface properties  $\text{H}_3\text{N}_2$

heparinised blood by treating with carbonyl iron, followed by differential centrifugation on Ficoll-Hypaque<sup>5</sup>. The purified lymphocyte suspension was washed twice with phosphate-buffered saline (PBS) and treated with formaldehyde<sup>6</sup> to prevent the formation of Fc and E rosettes with the indicator cells used later in the MAR. The fixed cells were washed twice with PBS and stored in sterile bottles at 4 °C until tested, at which stage they were washed twice more and resuspended to approximately  $2.5 \times 10^6 \text{ cells ml}^{-1}$  in 0.2% bovine serum albumin in PBS (PBS/BSA).

Preliminary experiments were carried out on blood lymphocytes from healthy individuals and the results compared with those on normal lymphocytes which had been

agent in NRS is antibody immunoglobulin which is able to combine with one or more antigenic components of influenza A. This conclusion was supported by further experiments in which the reactivity of NRS was retained, and actually increased, after treatment to remove nonspecific haemagglutination inhibiting factors<sup>7</sup> by (1) heating to 65 °C for 30 min. (2) treating with potassium periodate, (3) heating as in (1) plus periodate treatment, or (4) trypsin digestion.

The patients providing lymphocytes to be tested for *in vivo* adsorbed virus were selected by medical general practitioners on a clinical diagnosis of influenza. Between 24 h and 5 d after onset of symptoms, blood was taken, heparinised for lymphocyte separation, and clotted for

**Table 2** Percentages of reacting cells in MAR given by formalised normal human lymphocytes untreated, treated with 'split' influenza MRC11 strain virus\*, and treated with whole influenza MRC11 virus suspension†

Serum used in first stage of sensitisation	Lymphocytes untreated‡	Lymphocytes treated with split virus		Lymphocytes treated with whole virus (= 40 µg viral protein ml <sup>-1</sup> )
		(= 10 µg viral protein ml <sup>-1</sup> )	(= 1 µg viral protein ml <sup>-1</sup> )	
R9 anti-MRC11 1:25	2 (7)	98	61	93
1:100	3.5	99	49	95
R18 anti-MRC2 1:25	3 (6)	99	NT	98
1:100	1.5	98	NT	97
NRS pool 1:25	5 (5.5)	93	37	82
1:100	2	71	11	76
PBS/BSA control	1 (3)	13	3.5	23

\* Lymphocytes ( $2.5 \times 10^6 \text{ cells ml}^{-1}$ ) were mixed with split virus products, obtained by ether treatment of whole virus, at room temperature for 45 min. Cells were then washed twice before formaldehyde fixation.

† Whole virus purified from culture in allantoic fluid of hens' eggs was added to the lymphocyte suspension (room temperature 45 min) which was then washed and fixed as described above.

‡ Figures in parentheses are results for untreated control cells in test on lymphocytes exposed to whole virus.  
NT, Not tested.

exposed *in vitro* to either whole MRC11 strain (influenza recombinant  $\text{H}_3\text{N}_2$ ) virus suspension or ether-treated MRC11 split viral products (see footnote to Table 2). Lymphocytes from six normal individuals showed low percentages of reacting cells—usually 5% or fewer with either anti-influenza serum or NRS (Table 1). There were significant increases in percentages of rosetting lymphocytes, with up to 100% of cells reacting, when the lymphocytes were exposed to either whole MRC11 viruses or ether-treated split virus before formaldehyde fixation (Table 2). The reactivity, however, was also markedly increased, though to a somewhat lesser extent, in suspensions treated with NRS. A second pool of NRS and rabbit R9 pre-inoculation serum exhibited similar reactivity. When doubling dilutions of NRS (pool 23/4/70 and 30/6/70) and R18 anti-influenza serum were tested on lymphocytes coated with (1:100) split viral products, lower reactions were produced with the NRS, so that at 1:1,600 dilution R18 antiserum gave 92% rosettes while NRS gave only 31% weak rosettes. The reactive factor was also present in DEAE-cellulose purified rabbit IgG fraction, and  $\text{F(ab')}_2$  fragments of pepsin-digested rabbit IgG also reacted (Table 3). Since the uptake on to lymphocytes of rabbit serum proteins other than immunoglobulin would not be detected by the two-stage MAR, all the evidence suggests that the reactive

serum extraction. To confirm diagnosis, a throat swab was cultured and identification of the infecting agent attempted. A second serum sample was taken 10 d to 3 months after the onset, and both sera were tested by haemagglutination inhibition (HI) for any rise in anti-influenza A antibodies. The results of these tests are shown in Table 4.

Influenza A was grown from three of the ten patients and two more had serological evidence of infection. In four of the five positive patients the percentage of lymphocytes giving reactions for surface-bound influenza antigens

**Table 3** Percentages of reacting cells in MAR on normal human lymphocytes, untreated and treated with 1/20 diluted 'split' virus, to show reactions with NRS, DEAE-cellulose purified rabbit IgG and rabbit IgG pepsin  $\text{F(ab')}_2$ 

Reagent used in first stage of sensitisation	Dilution/concentration	Lymphocytes untreated	Lymphocytes treated with 1/20 split virus
NRS pool	1:25	7	85
NRS IgG	350 µg ml <sup>-1</sup>	7	89
NRS IgG $\text{F(ab')}_2$	320 µg ml <sup>-1</sup>	4	40
PBS/BSA control		2	11



**Table 4** Tests on patients with symptoms of influenza infection. Results of throat swab cultures, serum antibody titres measured by haemagglutination inhibition (HI) and percentages of lymphocytes in the MAR

Patient	Swab culture	Serum HI titre*	Mixed antiglobulin reaction of lymphocytes† following first stage sensitisation with		
			R9 anti-MRC11 (1/25)	R18 anti-MRC2 (1/25)	NRS pool (1/25)
R.B.	—	10 40	22	26	24
R.T.	+ ('A' Port Chalmers)	10 80	20	28	14
A.N.	+ ('A' Scotland)	10 1,280	45	NT	29
P.S.	No swab	10 80	19	16	12
P.H.	+ ('A' Port Chalmers)	<10 640	3	3	1.5
L.A.S.	—	<10 <10	2.5	1	1
J.M.	—	<10 <10	2	2	2
H.T.P.	No swab	20 20	9	4	4.5
J.D.A.	— (but + for respiratory syncytial virus)	160 160	4	7.5	6
J.J.B.	—	320 320	10	2	7

\* For each patient, the first figure is for serum collected at the same time as blood for lymphocytes, and the second for serum taken 10 d to 3 months later.

† These results were obtained 'blind', since the results of swab cultures and HI serum titres were unknown to the tester.

NT, Not tested.

was substantially greater than for the remainder of the group, in which the reactions were mainly similar to those of lymphocytes from normal, non-infected individuals. The highest proportion of reacting lymphocytes (45%) was found in a patient (A.N.) infected with influenza A Scotland 74 serotype, and he also showed the highest antibody titre (HI 1:1,280, 2 months after infection). Patient R. B., with 22% to 26% reacting lymphocytes, gave a negative culture, but the specific increase in serum HI titre (3 months postinfection) indicates that the infection was probably influenza.

The fifth influenza-positive patient (P.H.) gave a negative MAR test, although the virus had been grown and antibody production demonstrated. The lymphocytes from this person were obtained within 24 h of the onset of symptoms, whereas those from other positive patients were obtained 2–5 d after the onset. It is thus possible that there is a delay of at least 24 h between the onset of disease and the appearance of detectable viral antigen on circulating lymphocytes. It will be seen that, as with lymphocytes coated with viral antigens *in vitro*, lymphocytes from positive patients also reacted with NRS, although the numbers of rosetted cells found in three out of the four cases were lower than with anti-influenza serum.

The binding and subsequent inactivation of influenza and other myxoviruses by cultures of human and non-immunised, pathogen-free mouse lymphoid cells has previously been demonstrated<sup>8,9</sup>. Other viruses also known to adhere to the lymphocyte cell membrane are the Epstein-Barr virus, which attaches to B lymphocytes but not to T cells<sup>10</sup> and the measles virus, receptors for which are found only on T lymphocytes<sup>11</sup>. It would seem from the present work that influenza A will adhere *in vitro* to both T and B cells since < 100% of cells were positive in the MAR test. Fewer cells with attached virus were found in the peripheral blood of patients, however, and it is not yet known whether there is any bias in the *in vivo* adherence in favour of T or B cells.

Further investigations on influenza infection in ferrets have been initiated and it is hoped that these will provide information on the nature and spread of lymphocyte-adherent viral components in better controlled conditions than are possible in human infections.

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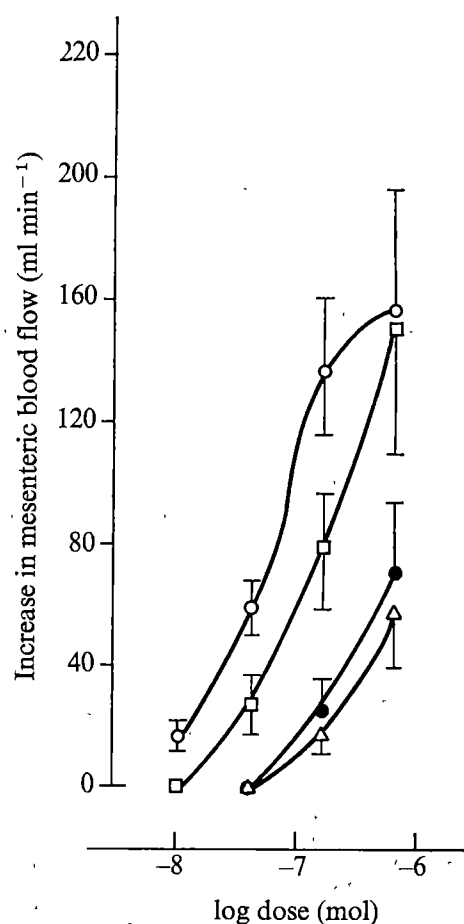
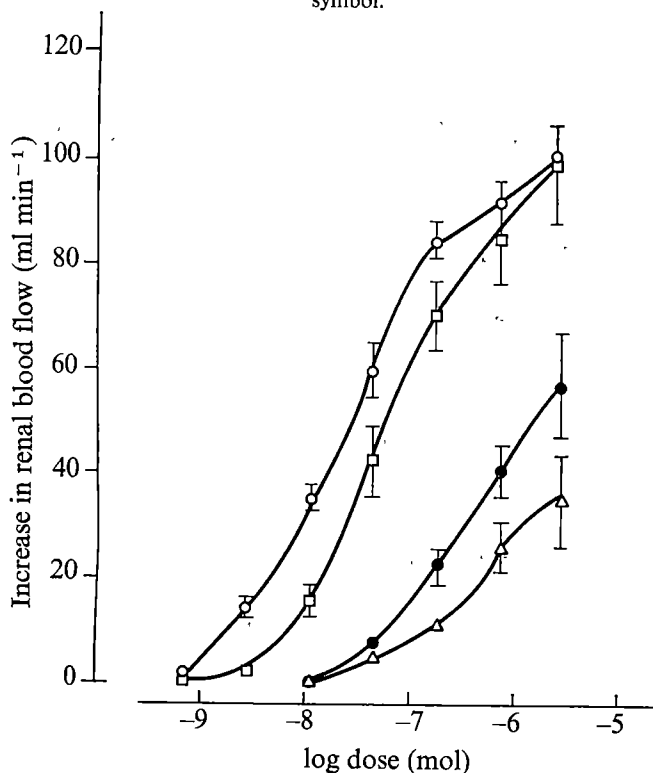
## Dopamine-like renal and mesenteric vasodilation caused by apomorphine 6-propylnorapomorphine and 2-amino-6, 7-dihydroxy-1,2,3,4-tetrahydronaphthalene

DOPAMINE has several unique actions in the central nervous system (CNS)—particularly the striatum—and in blood vessels, which seem to be due to interactions with specific receptors<sup>1,2</sup>. It is not known whether the vascular and CNS effects are mediated by actions on the same or different receptors. To demonstrate receptor identity, classical receptor theory requires that tissues respond in the same

rank order to a series of agonists of varying potency<sup>3</sup>. Remarkable similarities have been revealed in the relative efficacy of dopamine agonists in dilating canine renal blood vessels<sup>4</sup> and in increasing dopamine-sensitive cyclic AMP accumulation in homogenates of rat striatum<sup>5</sup>. Further characterisation has been restricted for lack of additional agonists to extend the potency series. We describe here a comparison of the actions of two recently described dopamine agonists, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) and 6-propyl-norapomorphine (PNA) with dopamine and apomorphine in the canine renal and mesenteric vascular beds. Apomorphine, which has structural similarities to dopamine, causes dopamine-like vasodilation<sup>4</sup> and increases cyclic AMP accumulation in rat striatum<sup>5</sup> but is much less potent and efficacious than dopamine in both systems. Woodruff *et al.*<sup>6</sup> found that ADTN exerted dopamine-like actions in the CNS of mice and resembled dopamine in decreasing blood pressure in guinea pigs. ADTN is equipotent with dopamine in increasing cyclic AMP accumulation in rat striatal homogenates<sup>5</sup>, and intra-striatal injections of ADTN in rats and guinea pigs produce a dyskinetic phenomenon characteristic of dopamine-like agents<sup>7,8</sup>. Cannon<sup>9,10</sup> suggested that PNA was a dopamine agonist on the basis of its emetic potency in the anaesthetised dog.

ADTN and PNA were chosen after preliminary experiments suggested that they have dopamine-like vasodilating actions. Methods were similar to those described before<sup>11</sup>. Mongrel dogs were anaesthetised with pentobarbital (32 mg kg<sup>-1</sup>) given intravenously. Anaesthesia was maintained by injections of 32 or 64 mg of pentobarbital when required. Blood flows in the renal, mesenteric and femoral arteries were monitored continuously by a Statham electromagnetic flowmeter coupled to a Beckman Dynograph Vasodilating

**Fig. 1** Mean increments in renal blood flow produced by intra-arterial injection of several doses of: ○, ADTN; □, dopamine; ●, PNA, and △, apomorphine. ADTN was compared with dopamine in five experiments; PNA and apomorphine were compared with dopamine in six more experiments. The dopamine curve represents the mean values obtained in all eleven experiments. Phenoxybenzamine (5 or 10 mg kg<sup>-1</sup>) was administered in the renal artery before injection of the vasodilators. Vertical lines indicate the s.e.m. when this value was larger than the symbol.



**Fig. 2** Mean increments in mesenteric blood flow produced by intra-arterial injections of several doses of: ○, ADTN; □, dopamine; ●, PNA, and △, apomorphine in five experiments in anaesthetised dogs. Phenoxybenzamine (5 or 10 mg kg<sup>-1</sup>) was administered intra-arterially before injection of vasodilators. Vertical lines indicate the standard error of the mean when this value was greater than the symbol.

agents were administered after intra-arterial injection of 5 or 10 mg of phenoxybenzamine, which was sufficient to eliminate or reverse the vasoconstricting actions of noradrenaline. Injections were made in a volume which caused no injection artefacts (0.2–0.4 ml). Dopamine, ADTN, PNA and apomorphine were administered in a fourfold geometric dose progression in doses ranging from  $3 \times 10^{-9}$  to  $3 \times 10^{-6}$  mol. The effects of ADTN and dopamine were investigated in one group of five dogs; in a separate group of six dogs, the effects of PNA were compared with both dopamine and apomorphine. In mesenteric blood flow experiments all agents were investigated in the same five dogs. In femoral blood flow experiments ADTN, dopamine and bradykinin were compared in one group of five dogs and PNA, apomorphine, bradykinin and dopamine in a separate group of five dogs. When large doses of the compounds were injected intra-arterially, recirculation usually had a subsequent effect on blood pressure. Only those changes preceding the alteration in blood pressure were considered in the analysis of the results.

The effects of various antagonists were investigated in two experiments with the renal vascular bed. (1) In five dogs, haloperidol ( $1.4 \times 10^{-7}$  mol) was injected into the renal artery simultaneously with the putative agonists. This dose of haloperidol causes transient selective attenuation of the vasodilation produced by dopamine<sup>12</sup>. (2) The agonists were administered intra-arterially to four dogs before and after intra-arterial injection of a solution containing propranolol (2 mg kg<sup>-1</sup>) and atropine (1 mg kg<sup>-1</sup>); these doses were sufficient to attenuate markedly the vasodilation produced by acetylcholine and isoproterenol. ADTN was synthesised

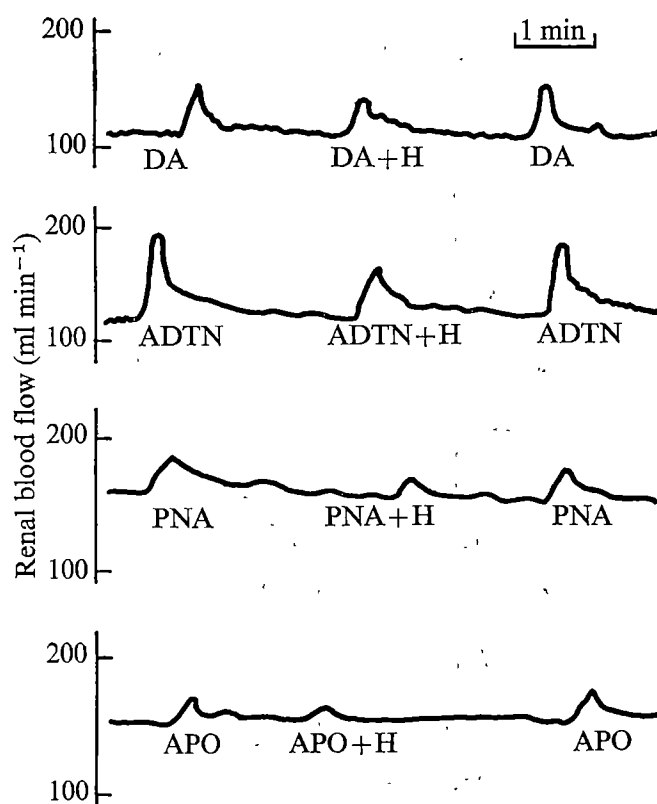


Fig. 3 Effects of injections of dopamine ( $4.7 \times 10^{-8}$  mol), ADTN ( $4.7 \times 10^{-8}$  mol), PNA ( $7.5 \times 10^{-7}$  mol), and apomorphine ( $7.5 \times 10^{-8}$  mol) alone and combined with haloperidol ( $1.4 \times 10^{-7}$  mol). Phenoxybenzamine ( $5$  or  $10$  mg  $\text{kg}^{-1}$ ) was administered in the renal artery before injection of vasodilators. Dopamine and ADTN were administered in one experiment; PNA and apomorphine in a different experiment

by R.M.P., PNA by W.B.H. Sources of the other compounds were as follows: dopamine HCl (Calbiochem), noradrenaline bitartrate, isoproterenol hydrochloride, bradykinin and acetylcholine (Sigma), phenoxybenzamine (Smith, Kline and French), propranolol HCl (Ayerst). The paired *t* test was used for statistical analyses.

Dopamine and ADTN increased renal blood flow in a dose-related manner (Fig. 1). In most experiments, equimolar doses of ADTN produced greater vasodilation than dopamine (Fig. 3) and the differences were statistically significant ( $P < 0.05$ ) at three of the doses studied ( $3 \times 10^{-9}$  mol,  $1.2 \times 10^{-8}$  mol,  $9 \times 10^{-7}$  mol). PNA and apomorphine were much less potent and effective than ADTN and dopamine. In most experiments, PNA produced greater renal vasodilation than apomorphine and the differences in the mean values were statistically significant for doses of  $1.9 \times 10^{-7}$  mol,  $7.5 \times 10^{-7}$  mol and  $3 \times 10^{-6}$  mol.

Similar responses were produced in mesenteric blood flow (Fig. 2). As in previous studies with dopamine, the dose-response curves were steeper than those constructed from experiments in the renal artery<sup>12</sup>. Again, ADTN was more effective than dopamine in most experiments and the difference was statistically significant at two doses,  $1.2 \times 10^{-8}$  mol and  $1.9 \times 10^{-7}$  mol. PNA was a more effective vasodilator than apomorphine in all experiments. But unlike the situation in renal experiments, the mean differences were not statistically significant at any dose.

Arterial blood pressure was not changed appreciably with smaller doses of the vasodilators. But with the highest two doses profound hypotension frequently occurred, making it difficult to estimate the maximum increase in renal and mesenteric blood flow. The hypotension was more pro-

nounced in the mesenteric experiments and thus data from the highest dose are not included in Fig. 2.

In contrast to the vasodilation produced by ADTN, PNA, apomorphine and dopamine in the renal and mesenteric vascular beds, femoral blood flow was not altered by these agents. As in previous studies<sup>11</sup>, bradykinin in doses ranging from  $1 \times 10^{-11}$  mol to  $5 \times 10^{-11}$  mol increased femoral blood flow in every experiment.

Haloperidol ( $1.4 \times 10^{-7}$  mol) caused transient attenuation of the renal vasodilating effects of ADTN, PNA and apomorphine in all experiments and at every dose (Fig. 3).

Combined administration of propranolol and atropine did not affect the renal vasodilation produced by ADTN, PNA or apomorphine. These agents, however, virtually abolished the vasodilation produced by isoproterenol in the dose range  $3 \times 10^{-8}$  mol to  $3 \times 10^{-7}$  mol and acetylcholine in the dose range  $5 \times 10^{-7}$  mol to  $3 \times 10^{-6}$  mol.

These experiments have demonstrated that ADTN, PNA and apomorphine satisfy the criteria established for *in vivo* characterisation of dopamine-like vasodilators<sup>2,13</sup>. First, after administration of phenoxybenzamine, dose-related vasodilation occurred in the renal and mesenteric vascular beds, but not in the femoral vascular bed. Second, vasodilation was not antagonised by propranolol and atropine but was attenuated selectively by haloperidol. Accordingly, the series of dopamine-like vasodilators has been extended to include more rigid cyclic compounds. In a previous investigation, apomorphine seemed to be dopamine-like, but haloperidol attenuation was not demonstrated<sup>4</sup>.

The greater efficacy of ADTN compared with PNA or apomorphine indicates that the preferred conformation for dopamine-induced vasodilation is the fully extended transoid form. A similar conclusion was reached<sup>8-9</sup> for putative dopamine receptors in the CNS. Indeed, the almost identical potency series of agonists causing dopamine-like effects in the blood vessels and in the brain, and the observations that phenothiazines and butyrophenones antagonise both central and vasodilating effects of dopamine support the suggestion that dopamine receptors in the two regions are identical<sup>1,2,14</sup>.

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## Possible involvement of central histamine H<sub>2</sub>-receptors in the hypotensive effect of clonidine

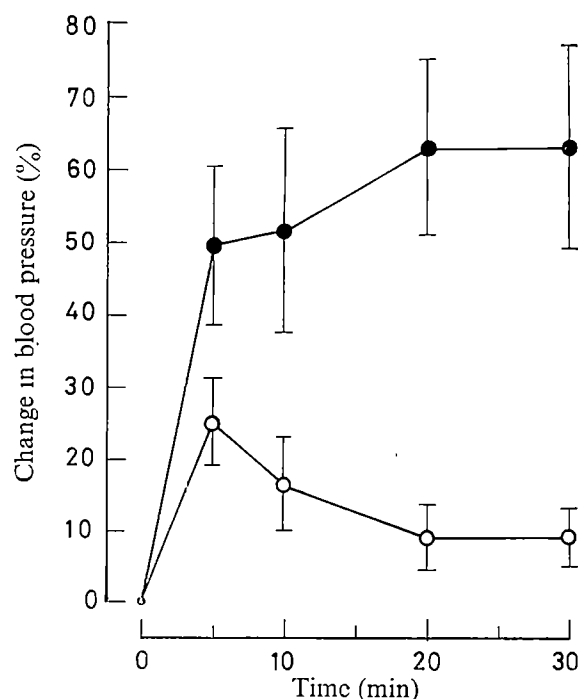
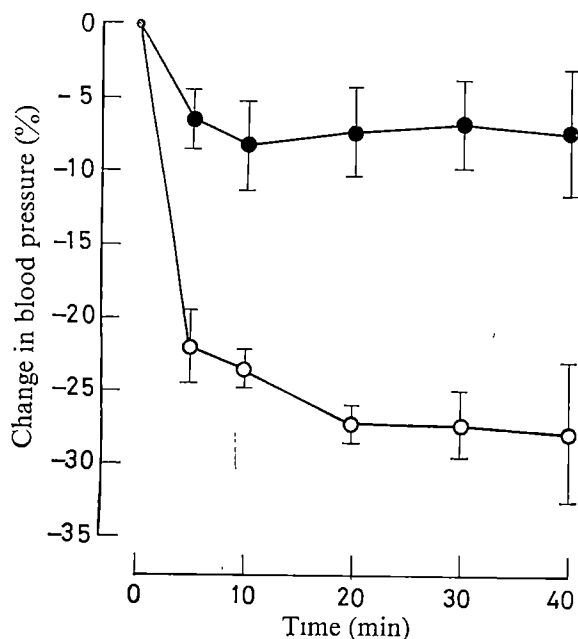
CLONIDINE and some other antihypertensive drugs exert their effect by acting on the central nervous system<sup>1-4</sup>. Some  $\alpha$ -adrenoceptor blocking drugs antagonise the centrally mediated hypotensive effect of clonidine<sup>5-7</sup>. It has therefore been suggested that clonidine acts by stimulating central  $\alpha$ -adrenoceptors, bringing about a depression of the cardiovascular system. The involvement of classical  $\alpha$ -adrenoceptors has, however, been challenged<sup>8,9</sup>. Moreover, in the brain tissue clonidine may even be a potent  $\alpha$ -adrenoceptor antagonist<sup>10</sup>.

We have previously reported that clonidine stimulates histamine H<sub>2</sub>-receptors in the gastric mucosa<sup>11</sup>, and recently it has been shown that clonidine also has the same effect in the heart<sup>12</sup>.

Histamine H<sub>2</sub>-receptors are present in the brain tissue<sup>13</sup>. Interestingly, alterations in the metabolism of brain histamine have been implicated in the pathogenesis of the spontaneous hypertension in rats<sup>14</sup>. We have now used the specific histamine H<sub>2</sub>-receptor antagonist, metiamide<sup>15</sup>, in order to study whether central histamine H<sub>2</sub>-receptors might be involved in the cardiovascular effects of clonidine.

Female Sprague-Dawley rats (200-250 g) were anaesthetised with urethane (1.5 g kg<sup>-1</sup> intraperitoneally). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously. The mean blood pressure was measured directly from the left femoral artery by means of a pressure transducer (Harvard apparatus 377). The heart rate was calculated from the pulse waves. Since

**Fig. 1** Influence of metiamide on the hypotensive effect of clonidine in rats. Metiamide (1.5  $\mu$ mol per rat) or saline was administered intracerebroventricularly, 10-15 min later clonidine (30  $\mu$ g kg<sup>-1</sup>) was injected intravenously. Differences between the metiamide (●) and control (○) groups were significant to the  $P < 0.001$  level for all measurements except that made at 40 min ( $P < 0.02$ ). The time elapsed after the injection of clonidine is shown on the abscissa. Vertical bars indicate s.e.m. The number of rats was 18 in the control group and 10 in the metiamide group.



**Fig. 2** Reversal of the clonidine-induced hypotension by metiamide. Clonidine (30  $\mu$ g kg<sup>-1</sup> intravenously) induced hypotension as shown in Fig. 1. 30 min after the injection of clonidine, metiamide (1.5  $\mu$ mol per rat) or saline was administered intracerebroventricularly. The time elapsed thereafter is shown on the abscissa. The differences between the metiamide (●) and control (○) groups were statistically significant at the  $P < 0.005$  level after 20 min, and the  $P < 0.01$  level after 30 min. Vertical bars, s.e.m. The number of rats was 9 in the control group and 6 in the metiamide group.

metiamide does not penetrate the blood-brain barrier, it was administered intracerebroventricularly.

In the first series of experiments, metiamide (1.5  $\mu$ mol per rat) was infused during 15 min into the lateral cerebral ventricle in a volume of 45  $\mu$ l (pH 6). Ten to fifteen minutes after the completion of the metiamide infusion, 30  $\mu$ g kg<sup>-1</sup> of clonidine hydrochloride was injected into the left femoral vein. The control rats were treated in exactly the same manner but received saline (pH 6) instead of metiamide solution.

The blood pressure values before the clonidine injections were  $110 \pm 4$  (s.e.) mmHg in the control rats and  $109 \pm 5$  (s.e.) mmHg in the metiamide-pretreated rats.

In the control rats clonidine induced a marked hypotension which was obvious for at least 40 min (Fig. 1). The maximum fall of blood pressure (approximately 30% from the initial level) was reached within 20 min and the blood pressure remained nearly constant for at least another 20 min. Metiamide alone did not affect the blood pressure. In the metiamide-pretreated rats, however, the hypotensive response to clonidine was markedly attenuated (Fig. 1) and the blood pressure remained for the whole 40-min observation period significantly higher ( $P < 0.02$  to  $P < 0.001$ ) than in the control rats. The bradycardiac effect of clonidine was slightly but not significantly ( $P > 0.05$ ) antagonised by the metiamide pretreatment.

In another series of experiments the ability of intracerebroventricular metiamide to reverse the clonidine-induced hypotension was studied. Thirty minutes after the intravenous injection of clonidine (30  $\mu$ g kg<sup>-1</sup>), 1.5  $\mu$ mol of metiamide was injected intracerebroventricularly within 2 min in a volume of 15  $\mu$ l. The control rats received 15  $\mu$ l of saline. Metiamide caused a clear elevation of the blood pressure level within 5 min (Fig. 2). The metiamide-induced increase of the heart rate was not statistically significant ( $P > 0.05$ ).

In contrast to metiamide, the histamine H<sub>1</sub>-receptor



blocking agent diphenhydramine (0.03 to 1.5  $\mu$ mol per rat intracerebroventricularly) did not antagonise the hypotensive effect of clonidine. The highest dose (1.5  $\mu$ mol per rat) was lethal to most of the rats.

The present results indicate that clonidine also stimulates histamine  $H_2$ -receptors in the central nervous system as it does in peripheral tissues<sup>11,12</sup>. Moreover, the findings also suggest that a stimulation of central histamine  $H_2$ -receptors brings about a depression of the cardiovascular system which is manifested as the lowering of blood pressure.

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## Induction of ovalbumin and conalbumin synthesis in immature chick oviducts by ethionine

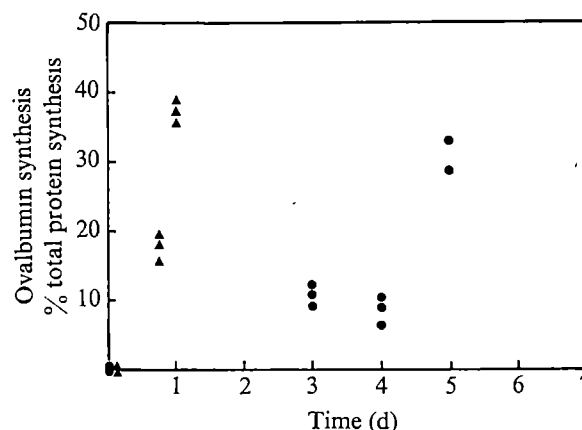
PROLONGED feeding of rats with ethionine results in a high frequency of hepatic carcinoma. Ethionine induces other pathological manifestations in other organs and tissues of experimental animals<sup>1</sup>, and some of its effects can be reversed rapidly by the administration of methionine. At the molecular level ethionine induces a rapid decrease in hepatic ATP concentration<sup>2-4</sup>, followed by inhibition of RNA<sup>5</sup> and protein synthesis<sup>6</sup>. The decrease in ATP concentration is due to formation of S-adenosyl-ethionine<sup>7</sup>, an inhibitor of tRNA methyltransferases<sup>8,9</sup>, and so tRNA isolated from the liver of rats injected with adenine and ethionine is hypomethylated<sup>10,11</sup>. It is well established that several eukaryotic mRNAs are methylated on the 5' terminus and that such modification is essential for translation<sup>12,13</sup>. To study the mechanism of methylation we attempted to produce a specific methyl-deficient mRNA by the administration of ethionine. To monitor the effectiveness of the deprivation of methyl groups the synthesis of hormone-induced ovalbumin in immature chick oviducts was followed. Administration of oestrogen to immature chicks causes cytodifferentiation and growth of the primitive oviduct<sup>14-17</sup>. The tubular gland cells synthesise ovalbumin, conalbumin, ovomucoid and lysozyme which comprise 85-90% of the egg-white proteins<sup>18</sup>. The con-

tinuous presence of oestrogen is required for sustained synthesis of these proteins in immature chicks; withdrawal of oestrogen is accompanied by a gradual decline in cell-specific protein synthesis as well as in the weight of the oviduct and the RNA content of the tissue<sup>17</sup>. But, the tubular gland cells in the oviduct magnum during withdrawal are retained although they do not synthesise cell-specific secretory proteins<sup>17,19</sup>. Readministration of hormone to chicks after withdrawal (secondary stimulation) results in restoration of cell-specific protein synthesis without concomitant need for DNA synthesis<sup>17</sup>. During these studies ethionine unexpectedly simulated the effects of the injection of oestrogen for secondary stimulation.

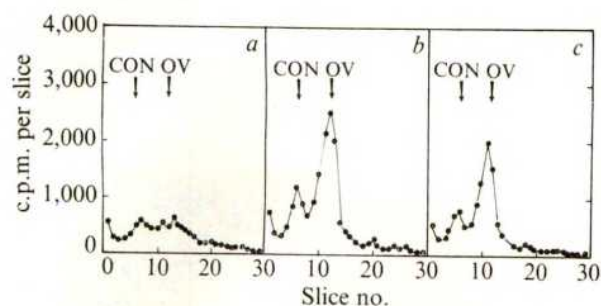
Four-day-old Calhoun chicks were injected with 1 mg of oestradiol benzoate in sesame oil for 10 d (ref 19). After a withdrawal period of 3-4 weeks the chicks were used in most of the experiments. The chicks were injected in groups of eight intraperitoneally with a 1-ml suspension in water of L-ethionine or methionine (0.25 mg per g body weight) and/or adenine (0.12 mg per g body weight) for 5-7 d. At the times specified the animals were killed and the capacity for ovalbumin synthesis of the magnum explants was determined. Oestradiol benzoate-17 $\beta$  was a product of Calbiochem, L-ethionine, adenine and methionine were from Sigma. <sup>14</sup>C-amino acid mixture (algal profile) was purchased from Schwarz/Mann. Medium 199 ( $\times 10$ ) and NaHCO<sub>3</sub>-free Hanks balanced salt solution ( $\times 10$ ) were from Grand Island Biological. Anti-ovalbumin and conalbumin sera were elicited in rabbits.

Chicks were decapitated and their oviducts were removed aseptically, the magnum portions of the oviduct were freed of adjoining tissue and were cut into small pieces. The magnum explants were incubated *in vitro* in antibiotic-free medium<sup>20-22</sup>. Tissue (100-200 mg) was incubated in 2 ml of medium in 25-ml, rubber stoppered Erlenmeyer flasks. Each flask contained tissue from at least three oviduct magna. The incubations were carried out at 37 °C for 3 h with constant shaking and the flasks were gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> at hourly intervals. At the end of the incubation the pieces of tissue were blotted on filter paper, homogenised in 2 ml of 15 mM NaCl-10 mM sodium phosphate, pH 7.5, and centrifuged at 100,000g for 1 h. The high speed supernatant was used for subsequent analyses<sup>20</sup>. Labelled ovalbumin was determined essentially according to Rhoads *et al.*<sup>23</sup>. The tubes were incubated at 37 °C for 1 h and then stored at 4 °C until they were filtered. The precipitates were collected on Millipore HAWP filters, 0.45  $\mu$ m (pre-wetted with the wash solution) and were washed three times

**Fig. 1** Time course of induction of ovalbumin synthesis in immature chick oviducts by oestradiol ( $\blacktriangle$ ), and ethionine and adenine ( $\bullet$ ). Chicks withdrawn from primary stimulation for 4 weeks were injected with oestradiol (1 mg) or ethionine and adenine as described in the text. At various times five chicks were killed and ovalbumin synthesis was determined in duplicate or triplicate samples. Details are the same as for Table 1.







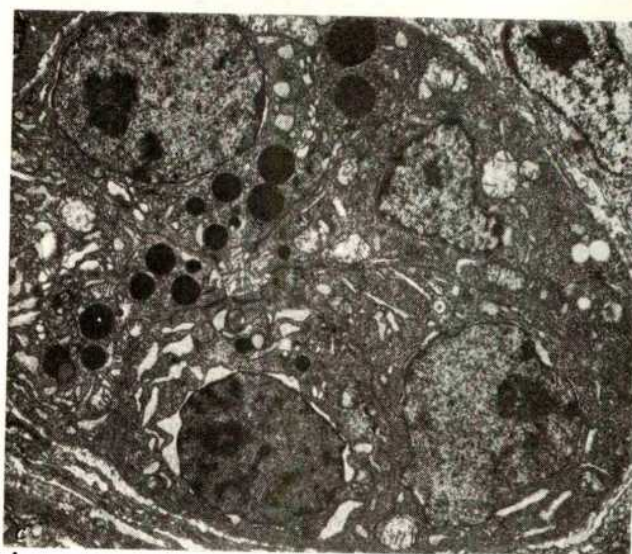
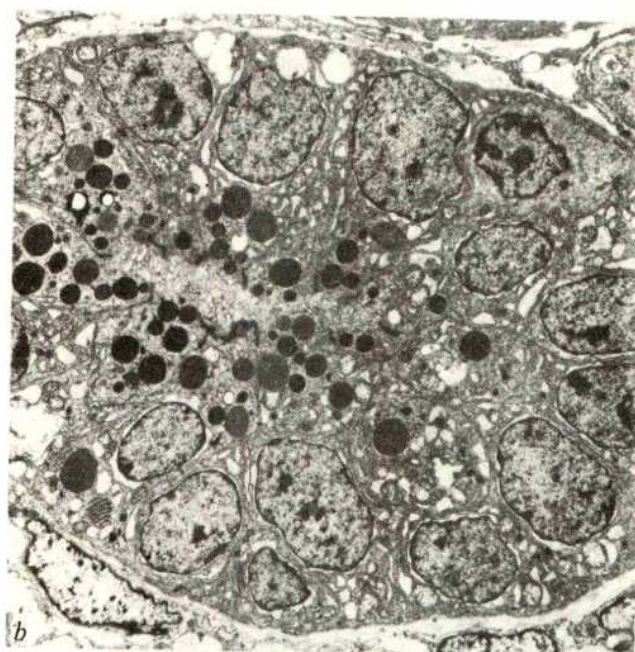
**Fig. 2** SDS-acrylamide gel electrophoresis of high speed supernatants from incubations. Oviduct explants were incubated as described in the text. Samples of 50–100  $\mu$ l of the high speed supernatant were precipitated with trichloroacetic acid and electrophoresed on SDS-acrylamide gels<sup>20</sup>. The migration of conalbumin (CON) and ovalbumin (OV) is indicated. *a*, Chicks withdrawn for 28 d, after 10 d of primary oestrogen stimulation; *b*, withdrawn chicks injected with oestrogen for 24 h; *c*, withdrawn chicks injected with ethionine and adenine for 5 d.

with 1-ml samples of 10 mM sodium phosphate, pH 7.5 containing 150 mM NaCl, 1% deoxycholate and 1% Triton X-100. The filters were dried at 100 °C for 30 min and counted in a liquid scintillation counter. The reactions were performed so that at least three concentrations of ovalbumin precipitated in the linear range (antibody excess). A blank of bovine serum albumin (5  $\mu$ g) and rabbit anti-bovine serum albumin was used to correct for non-specific precipitation<sup>24</sup>.

When, 4 weeks after primary oestrogen stimulation—at which time oviducts do not synthesise ovalbumin—ethionine and adenine were injected for 3 d followed by secondary oestrogen stimulation with oestradiol for 18 h, the outcome was unexpected. Ovalbumin synthesis in these chicks was greater than in those which received oestradiol alone (data not shown). Therefore, the effect of the administration of ethionine without secondary oestrogen stimulation was studied in chicks 4 weeks after primary stimulation (Table 1).

Ethionine itself induced ovalbumin synthesis although a combination of ethionine and adenine was more effective. Adenine alone and in combination with methionine did not induce ovalbumin synthesis. The induction of ovalbumin synthesis by ethionine was slower than by oestrogen (Fig. 1). Ethionine is highly toxic to chicks and the animals died after 5–7 d. Conalbumin synthesis was also induced by ethionine as measured by precipitation with monospecific antiserum to conalbumin (data not shown). Induction of ovalbumin and conalbumin synthesis was confirmed by sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis (Fig. 2).

Electron microscopy of the oviduct revealed in the control tissues well developed tubular glands but the cytoplasm of the glandular cells was devoid of the organelles associated with specific intracellular synthesis (Fig. 3*a*). In the tissues of ethionine-treated chicks the nuclei of the glandular cells were located basally, the rough endoplasmic reticulum and the Golgi complex were well developed, electron-dense secretory granules were prominent and the



**Fig. 3** Electron micrographs of chick oviduct magna. The excised oviduct magnum tissue was minced into 1 mm cubes, fixed in buffered 4% glutaraldehyde for 1 h, postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite via propylene oxide. Thin sections were stained with hot alcoholic uranyl acetate<sup>25</sup> and lead citrate<sup>26</sup>. *a*, Tubular gland from control chicks, the nuclei are randomly distributed and the cytoplasm has a minimal number of organelles ( $\times 6,250$ ). *b*, Tubular gland from ethionine-treated chicks; the nuclei are basal; the cytoplasm contains abundant rough endoplasmic reticulum, Golgi complex and apical-secretory granules ( $\times 6,500$ ). *c*, Tubular gland from oestradiol-treated chicks, the cytoplasmic organelles are even more prominent and there are abundant ribosomes ( $\times 6,750$ ).



Table 1 Induction of ovalbumin synthesis in immature chick oviducts by ethionine

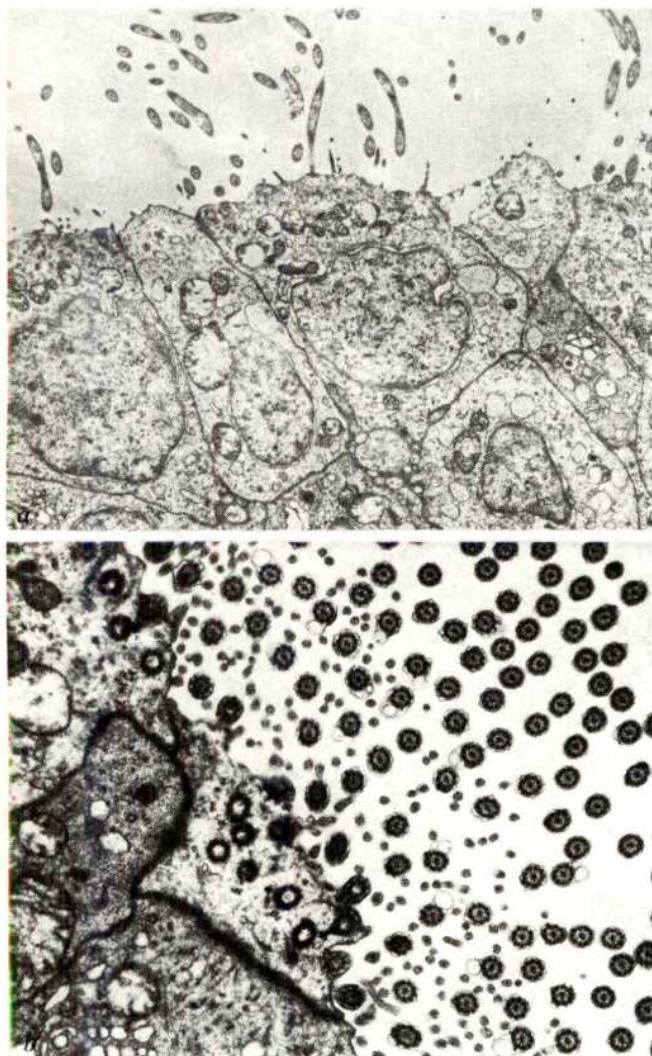
Treatment	Oviduct magnum* weight per chick (mg)	<sup>14</sup> C-amino acids incorporated per mg of soluble protein (c.p.m. $\times 10^{-5}$ )	<sup>14</sup> C-amino acids incorporated into ovalbumin per mg of soluble protein (c.p.m. $\times 10^{-5}$ )	Ovalbumin synthesis, % of total protein synthesis
None	230 (4)	0.77 0.85	Not detected	
Oestradiol†	580 (4)	0.83 0.98	0.354 0.369	42.7 37.7
Ethionine	260 (8)	1.53 1.14	0.384 0.108	25.1 9.5
Ethionine+ adenine	300 (7)	1.42 1.23	0.498 0.598	35.1 48.6
Adenine	180 (8)	1.10 1.01	Not detected	
Adenine+ methionine	230 (8)	0.77 0.85	Not detected	

After 10 d of primary oestrogen stimulation chicks were withdrawn for 28 d and in groups of eight were injected intraperitoneally for 5 d as described in the text. On day 6 the animals were killed and ovalbumin synthesis in the oviduct magnum explants was determined as described. The incubations were carried out in duplicate and the flasks contained tissue from at least two different chicks.

\*Average magnum weight, the number in parentheses refers to the number of chicks.

†Secondary oestrogen stimulation was by 1 mg of oestradiol for 24 h.

Fig. 4 Electron micrographs of epithelia of chick oviduct magna. Tissue processing was identical to that described for Fig. 3. Surface epithelium from control chicks (a), the epithelial cells contain sparse cytoplasmic organelles, few cilia are present in the surface ( $\times 5,000$ ). b, Surface epithelium from ethionine-treated chicks. The cells contain abundant cytoplasmic organelles and cilia are prominent in the oviduct cavity ( $\times 6,500$ ).



cells had membrane specialisation resembling tight junctions, desmosomes and microvilli (Fig. 3b). In the cells of oestradiol-treated oviduct, the nuclei were basal, and the rough endoplasmic reticulum and Golgi complexes were even better developed than in the tissue of ethionine-treated animals. The secretory granules were more prominent, membrane differentiation was obvious and the cytoplasm contained abundant ribosomes (Fig. 3c). A process similar to that in the glands, was obvious in the surface epithelium<sup>27,28</sup>. The surface cells in the controls had few organelles, only occasional cilia and sparse membrane specialisation. In the tissues of the animals exposed to oestradiol or ethionine, cell organelles, membrane specialisation and cilia became abundant (Fig. 4).

Induction of ovalbumin and conalbumin synthesis by ethionine was not observed in the "primitive oviduct" of chicks (5–6 weeks old) which had not received primary stimulation with oestrogen. This suggests that ethionine alone cannot cause cytodifferentiation and growth of the primitive oviduct, but that in tissues already differentiated by the action of oestradiol it can induce the further cellular differentiation needed for specific protein synthesis. The mechanism of induction of protein synthesis by ethionine remains obscure: it may or may not stem from interference with the methylation of either mRNA or tRNA or both. In view of the powerful oncogenicity of the agent the phenomenon is, however, worthy of study to delineate the mechanism of its induction.

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## Regulation of degradation of thyrotropin releasing hormone by thyroid hormones

WHEN thyrotropin releasing hormone (TRH, pyroGlu-His-Pro-NH<sub>2</sub>) was isolated and its structure determined<sup>1,2</sup> the concept of neurohumoral regulation of hormone secretion by the anterior pituitary<sup>3</sup> was confirmed, but the mechanisms involved are not well understood. Enzymatic inactivation of TRH by blood and serum may regulate the hormone's activity by determining the number of molecules available to the thyrotroph<sup>4</sup>. With this physiological function, degradative enzyme(s) would itself need to be regulated and I report here that the activity of the TRH-degrading enzyme(s) is controlled by the thyroid hormones.

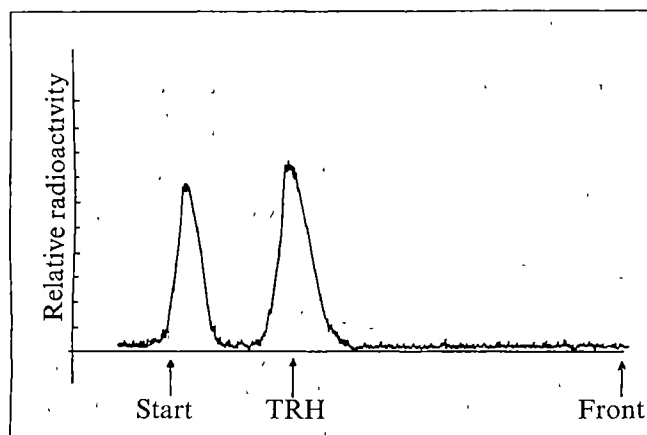
Rapid inactivation of TRH by serum was reported before its structure was elucidated<sup>5,6</sup>, but the mechanism of degradation remains to be elucidated. Based on the observation that the free amino acids are the main degradation products<sup>7</sup>, a quantitative test of TRH-degradation was developed. After incubation of TRH (labelled with <sup>3</sup>H in the histidine moiety) with serum, the labelled products

could be separated by ion exchange chromatography as described in the legend of Fig. 1. TRH migrated with an *R<sub>f</sub>* of 0.25 as identified by cochromatography of synthetic material which was visualised using Pauly reagent. All labelled degradation products remained absorbed near the origin, as shown by rechromatography of the material from the TRH zone with standard peptides. Thin-layer chromatography in several solvent systems and electrophoresis at pH 1.9 and 6.4, revealed one radioactive spot comigrating with the standard hormone while all the possible labelled cleavage products migrated separately (Table 1). Radioactivity in the TRH zone was determined by scintillation counting.

This test provided a simple and sensitive method for quantitative evaluation of TRH degradation by serum (see legend to Fig. 2). It is thus possible to follow the kinetics of TRH degradation by serum and to determine the initial rate as a measure of enzyme activity. In the conditions described (Fig. 2), the initial rate of degradation increased from 1.6% min<sup>-1</sup> for serum of untreated rats to 3.3% min<sup>-1</sup> for that of hyperthyroid animals (injected with triiodothyronine, T<sub>3</sub>). With serum from hypothyroid rats (treated with propylthiouracil, PTU) degradation was very slow (0.6% min<sup>-1</sup>) but restored to above normal (1.7% min<sup>-1</sup>) within 72 h by daily injection of 10 µg T<sub>3</sub>. This observation parallels the reported reduced TRH inactivation of plasma from hypophysectomised-thyroidectomised rats which was restored by treatment with T<sub>3</sub> (ref. 8). Because of the limitations of the biological assay, these studies could not be confirmed<sup>9</sup> and have not been extended.

With the demonstration of a single exponential relationship of TRH degradation with time (Fig. 2) and serum protein (our unpublished results) degradation activity could be determined by a four-point assay with four serum dilutions. When the degradation activity of serum from untreated control animals was compared with that from animals injected with T<sub>3</sub> or treated with PTU, the effect of the thyroid hormones could be demonstrated clearly (Fig. 3). As Fig. 3a shows, the activity of the serum enzyme(s) declined slowly when the formation of thyroid hormones

**Fig. 1** Separation of labelled split products from TRH-<sup>3</sup>H-histidine incubated with serum. Trunk blood from rats was collected in plastic tubes and allowed to clot for 3 h at 0 °C. Serum was obtained by centrifugation at 5,000 r.p.m. for 15 min. Serum (10 µl) diluted 1:3 with buffer A (100 mM Tris-HCl pH 7.6, 1 mM DTT), was incubated at 37 °C for 30 min with 1.0 µCi of purified TRH-<sup>3</sup>H-histidine (18 Ci mmol<sup>-1</sup>) in 20 µl buffer A. The reaction mixture and 10 µg synthetic TRH were applied to prewashed cellulose phosphate paper which was then developed with 1 N acetic acid. The labelled products were localised by scanning for radioactivity (Berthold Scanner, LB 2723) and the cochromatographed TRH was stained with the Pauly reagent.

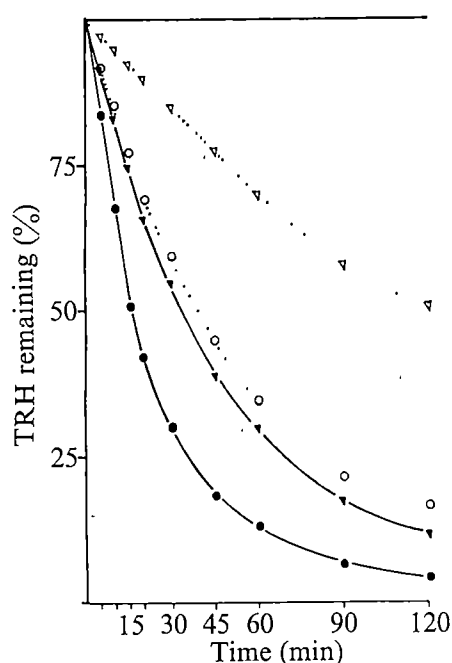


**Table 1** Chromatographic separation of TRH from cleavage products

Thin-layer chromatography in solvent system	<i>R<sub>f</sub></i> of substance					
	1	2	3	4	5	6
A	0.57	0.36	0.21	0.58	0.30	0.14
B	0.63	0.5	0.46	0.40	0.27	0.21
C	0.56	0.48	0.37	0.43	0.35	0.19
Paper electrophoresis at	Migration (cm)					
	pH 1.9	pH 6.4	pH 6.5	pH 32	pH 24.5	pH 24.5
pH 1.9	14.5	13.5	14.5	30	24.5	24.5
pH 6.4	20.5	6.4	6.5	32	21.5	25.5

The material from the TRH corresponding zone of Fig. 1 was eluted with 0.5 ml 1 N ammonia using extraction thimbles (Reeve Angel) and the standard material was applied to silica gel precoated plates (Merck No. 5721) for thin-layer chromatography or to paper (Whatman 3M) for electrophoresis. 1, TRH; 2, deamido-TRH; 3, pyroGlu-His; 4, His-Pro-NH<sub>2</sub>; 5, His-Pro; 6, His. The plates were developed in vapour-saturated tanks using the solvent systems: A, CHCl<sub>3</sub>-CH<sub>3</sub>OH-NH<sub>3</sub>aqueous (125:75:25); B, propanol-methyl-ethylketone-pyridine-acetic acid-water (40:40:40:20:40); C, phenol-water (150:50). Paper electrophoresis was carried out at 50 V cm<sup>-1</sup> using buffer pH 1.9 (HCOOH-CH<sub>3</sub>COOH-H<sub>2</sub>O 50:150:800) or buffer pH 6.4 (pyridine-acetic acid-water 100:10:890). The labelled material was localised by scanning for radioactivity and the cochromatographed standard material by staining with Pauly reagent or by the chlorine-iodide reaction for the phenol-containing solvent system.





**Fig. 2** Time course of degradation of TRH by serum. 0.5  $\mu$ Ci of TRH- $^3$ H-histidine in 40  $\mu$ l of buffer was incubated at 37°C with 20  $\mu$ l of serum (diluted 1:3 with buffer A). At various times 5  $\mu$ l of the reaction mixture was spotted on the ion exchange paper. After development in 1 N acetic acid the strips were segmented and the segments corresponding to the TRH zones were placed in scintillation vials. 500  $\mu$ l 2 N ammonia per segment (1.5  $\times$  2 cm) was added to each vial and after 10 min 10 ml of Bray solution was added before scintillation counting. The values are expressed as percentage of the total TRH added. The serum was obtained, as for Fig. 1, from one group of male rats, 6 months old.  $\circ$ , Untreated;  $\bullet$ , 10  $\mu$ g  $T_3$  per 100 g body weight given subcutaneously every 24 h, 72 h before decapitation;  $\nabla$ , PTU (200 mg  $l^{-1}$ ) was added to the drinking water for 4 months;  $\blacktriangledown$ , PTU-pretreated rats were injected with  $T_3$  as before.

was blocked by the goitrogenic agent PTU. In the conditions described (Fig. 3) peptidase activity was reduced by 50% after 40 d. Whether the slow decline reflects the long half life of the circulating enzyme(s) needs further investigation.

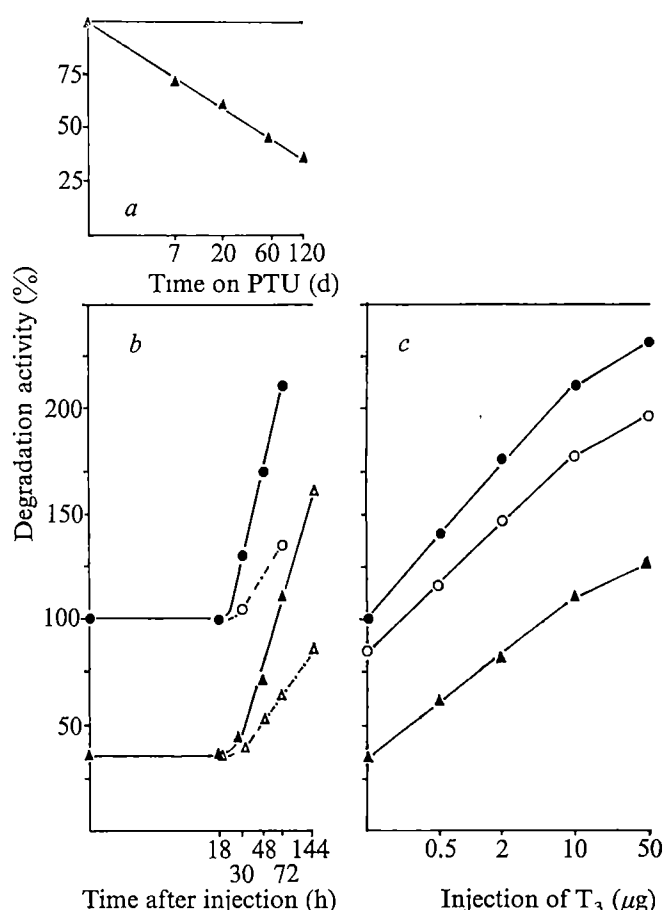
In contrast, a rapid increase of enzymatic activity became evident 24 h after injection of normal and hypothyroid animals with  $T_3$  (Fig. 3b): 72 h after injection there had been a 2.1-fold increase of enzymatic activity for euthyroid animals. The rate of increase was greater for euthyroid animals than for those pretreated with PTU. In parallel with biological activity, the stimulation of the enzyme activity was less pronounced when thyroxine ( $T_4$ , Sigma) was injected, but the onset of hormone expression was the same. The prolonged lag period and the preliminary observation that stimulation is inhibited by actinomycin D or puromycin indicate that the appearance of enzymatic activity is preceded by the induction of protein (enzyme) synthesis, as with the induction of hepatic cellular activities by thyroid hormones<sup>9</sup> or feedback control of pituitary thyroid stimulating hormone (TSH) secretion by  $T_3$  or  $T_4$  (refs 10–12).

As Fig. 3c shows, the effect of the thyroid hormones is strongly dose related. Even after subcutaneous injection of only 0.5  $\mu$ g  $T_3$  per 100 g body weight per day, there was strong stimulation of enzymatic activity. Euthyroid female rats showed only 85% of the enzymatic activity of euthyroid males. The origin of this sex difference is as obscure as the sex difference of plasma TSH and  $T_4$  in rats<sup>13</sup>. The response to  $T_3$  was less for female and PTU-treated rats than for control males.

These results show that the activity of the TRH-

degrading enzyme(s) is controlled by the thyroid hormones. Because there is evidence that blood collected from the hypothalamo-hypophyseal portal vessels inactivates TRH (ref. 6 and R. L. Eskay, personal communication), my results suggest that the regulation of the positive stimulus of TRH on the thyrotroph could be the physiological function of these enzyme(s). The enzymatic degradation would thus contribute to the balance of thyrotrophin secretion, which in turn is controlled by the thyroid hormone-induced negative feedback mechanism acting directly on the thyrotroph<sup>10–12</sup>. This sensitive and rapid assay provides a tool for further studies of the implication of the enzymatic degradation of TRH in the regulation of the hypothalamic-pituitary-thyroid axis, in clinical diagnosis (TRH-induced TSH-secretion tests) and in pathological disorders (enzyme defects) which might contribute to the "inappropriate secretion of TSH".

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**Fig. 3** Effect of thyroid hormones on the degradation of TRH activity of serum. Serum was obtained as for Fig. 1 and assayed at four dilutions (1:1, 1:2, 1:3 and 1:4) following a four-point kinetic (0, 7.5, 15 and 30 min), as described in Fig. 2. Degradation activity is expressed as percentage of the enzyme activity of serum from untreated male rats kept as control animals. **a** TRH degradation activity of serum from male rats after treatment with PTU (200 mg  $l^{-1}$  drinking water) for various times. **b**, At given times serum was obtained from 6-month-old male rats, injected subcutaneously with 10  $\mu$ g  $T_3$  or  $T_4$  per 100 g body weight every 24 h.  $\bullet$ , Untreated, injected with  $T_3$ ;  $\circ$ , untreated injected with  $T_4$ ;  $\blacktriangle$ , pretreated with PTU (200 mg  $l^{-1}$  drinking water) for 4 months, injected with  $T_3$ ;  $\triangle$ , PTU-pretreated, injected with  $T_4$ . **c**, TRH degradation activity of serum from 6-month-old rats injected subcutaneously every 24 h with the indicated dose of  $T_3$  per 100 g body weight 72 h before decapitation.  $\bullet$ , Males;  $\circ$ , females;  $\blacktriangle$ , males pretreated with PTU as before for 4 months.

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## Primer-dependent copying of rabbit globin mRNA with Q<sub>β</sub> replicase

THE synthesis of complementary strands of heterologous RNAs has been achieved by incubating these RNAs with Q<sub>β</sub> replicase in the presence of Mn<sup>2+</sup> (refs 1, 2). Q<sub>β</sub> replicase has been used in this way for the *in vitro* copying of RNAs for which no replicating enzymes are available. A further extension of such synthesis is offered by the recently discovered primer-dependent RNA synthesis by Q<sub>β</sub> replicase<sup>3</sup>. This approach is specially suited for the copying of eukaryotic RNAs (mRNAs) with a 3'-terminating poly(A) sequence. The complementary RNA synthesis is started at these poly(A) sequences with oligo(rU)<sub>6</sub> as primer and continues into the heterologous part of the mRNAs. The particular advantages of this method are the independence of special structural features of the template RNA for the initiation of synthesis and the possibility to inhibit completely any disturbing 6S RNA synthesis which normally takes place in Q<sub>β</sub> replicase incubations. Because of its defined start of synthesis, the newly synthesised complementary RNA may be very useful for sequence determinations of the template RNAs. I describe here experiments using rabbit globin mRNA as an example of the primer-dependent copying of eukaryotic mRNAs.

The standard conditions for these replicase incubations were as follows: Q<sub>β</sub> replicase (preparation described in ref. 6) 80 U ml<sup>-1</sup>, 12 mM MgCl<sub>2</sub>, 80 mM Tris-HCl (pH 7.6), 0.8 mM ribonucleoside triphosphates, and rabbit globin mRNA (Searle) as well as oligo(rU)<sub>6</sub> (Boehringer) in concentrations as specified. Since endogeneous 6S RNA synthesis is a major problem in Q<sub>β</sub> replicase incubations with heterologous templates in the presence of the four common ribonucleoside triphosphates (ATP, UTP, CTP, GTP) (refs 4 and 5), conditions were sought which would inhibit this synthesis. 6S RNA synthesis is strictly dependent on the presence of GTP and it can be avoided completely if GTP is replaced by ITP in the incubation mixture (data not shown). This inhibition of 6S RNA seems to involve the initiation step, since the substitution of GTP by ITP does not interfere with the elongation steps of Q<sub>β</sub> replicase reactions<sup>3</sup>. As the primer-dependent start of RNA synthesis is independent of GTP, GTP was replaced by ITP in the following enzyme assays, thus inhibiting the concomitant 6S RNA synthesis.

The kinetic analysis depicted in Fig. 1 shows that in such enzyme incubations the incorporation of <sup>3</sup>H-CMP and <sup>3</sup>H-AMP in response to globin mRNA as template is greatly stimulated

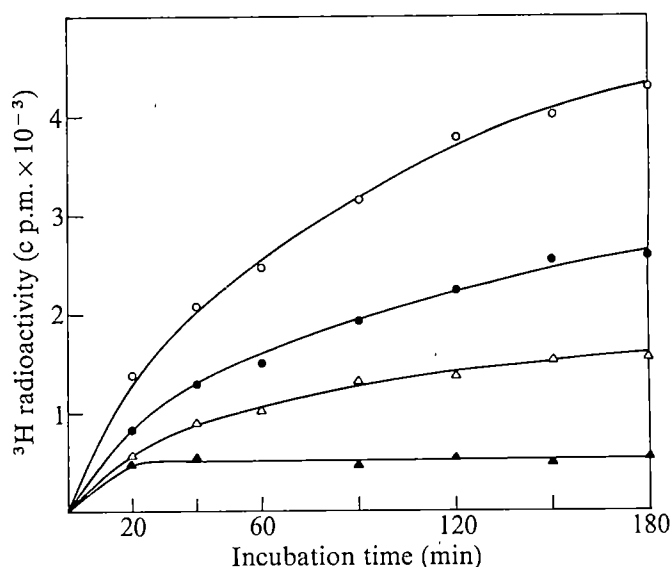


Fig. 1 Kinetics of oligo(rU)<sub>6</sub>-dependent copying of rabbit globin RNA. Standard incubation mixtures (60 µl) containing <sup>3</sup>H-CTP (7 × 10<sup>4</sup> c.p.m. nmol<sup>-1</sup>) and <sup>3</sup>H-ATP (7 × 10<sup>4</sup> c.p.m. nmol<sup>-1</sup>), 40 µg ml<sup>-1</sup> rabbit globin RNA and no oligo(rU)<sub>6</sub> (Δ), 70 µg ml<sup>-1</sup> oligo(rU)<sub>6</sub> (△), 140 µg ml<sup>-1</sup> oligo(rU)<sub>6</sub> (●), or 250 µg ml<sup>-1</sup> oligo(rU)<sub>6</sub> (○) were incubated at 30 °C. At the times indicated samples were taken (4 µl) and analysed for 6% trichloroacetic acid-insoluble radioactivity as described previously<sup>6</sup>.

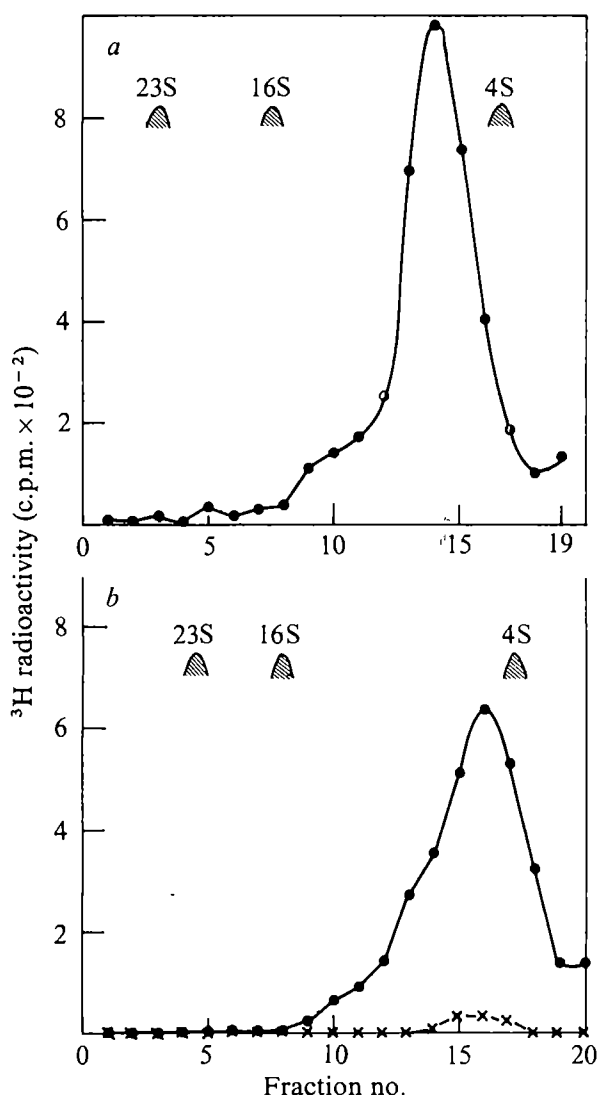
by high concentrations of oligo(rU)<sub>6</sub>. Incubations with <sup>3</sup>H-CTP as the only radioactive substrate give the same result. It should be stressed that in addition to the great molar excess of primer, the template-enzyme ratio is also important for a successful synthesis. Incubations without template resulted in less than 5% of the RNA synthesis obtained in assays with template.

To ascertain the amount of globin-specific RNA in the total reaction product, renaturation tests were carried out after denaturation of the product. Table 1 shows that in the case of the product from a 3-h incubation, 49% of the incorporated radioactivity can be renatured to a double-stranded structure as analysed by RNase digestion in standard conditions. Some experiments yielded up to 80% of this double-stranded RNA. Addition of an excess of globin mRNA to the renaturation

Table 1 Renaturation of product RNA with template RNA

Sample	Time of annealing (min)	Resistance to RNase (%)
1 Non-denatured	0	51
2 Denatured	0	4
3 Denatured	5	28
4 Denatured + 25 µg ml <sup>-1</sup> globin mRNA	5	42
5 Denatured + 125 µg ml <sup>-1</sup> globin mRNA	5	45
6 Denatured + 125 µg ml <sup>-1</sup> Q <sub>β</sub> RNA	5	22
7 Denatured	30	36
8 Denatured + 125 µg ml <sup>-1</sup> globin mRNA	30	49

A 100-µl standard incubation mixture containing <sup>3</sup>H-CTP (1 × 10<sup>5</sup> c.p.m. nmol<sup>-1</sup>) and <sup>3</sup>H-ATP (1 × 10<sup>5</sup> c.p.m. nmol<sup>-1</sup>), 40 µg ml<sup>-1</sup> globin RNA and 250 µg ml<sup>-1</sup> oligo(rU)<sub>6</sub> was incubated for 3 h at 32 °C, then brought to 1% SDS and 20 mM EDTA, phenolised, dialysed against 0.1 mM EDTA and concentrated to a small volume by desiccator evaporation. Aliquots containing 3,000 c.p.m. <sup>3</sup>H-product were supplemented with additional RNAs as shown and heated for 1.5 min at 100 °C. The samples were then adjusted to 0.75 M NaCl and 0.075 M Na-citrate in a total volume of 20 µl and annealed for the times indicated at 65 °C. After diluting the samples to 2 ml with 1 × SSC, the solutions were digested by RNase in standard conditions<sup>7</sup> and analysed for acid-precipitable radioactivity<sup>6</sup>.



**Fig. 2** Sedimentation analysis of the enzymatic product before and after denaturation. The SDS-treated and phenolised product of an incubation as described in Table 1 was adjusted to 0.5 M NaCl, heated for 5 min at 65 °C, diluted with and dialysed against the appropriate buffer for subsequent chromatography on a small cellulose column according to Franklin<sup>8</sup>, omitting the 30% ethanol step. The RNA-containing fractions eluted with TSE buffer<sup>8</sup> (analysed by trichloroacetic acid precipitation of aliquots) were pooled, dialysed against 0.1 mM EDTA and concentrated to a small volume by desiccator evaporation.  $^{32}\text{P}$ -labelled *E. coli* RNA was added and 20  $\mu\text{l}$  of this solution layered directly (a) on a 5–23% linear sucrose gradient (0.1 M NaCl, 0.005 M EDTA, 0.05 M Tris-HCl pH 7.6). In b 30  $\mu\text{l}$  of solution was diluted to 60  $\mu\text{l}$  with  $\text{H}_2\text{O}$ , heated first for 1 min at 100 °C and quickly cooled before layering on a gradient. After centrifugation for 4 h at 64,000 r.p.m. in the SW 65 rotor of the Beckman centrifuge, the  $^3\text{H}$ - and  $^{32}\text{P}$ -radioactivities of the fractions were counted after acid precipitation. One part of the fractions of b was treated with RNase in standard conditions before the acid precipitation and counting ( $\times$ ); the other part was analysed directly ( $\bullet$ ).

mixture speeded up the renaturation, but the presence of  $\text{Q}_\beta$  RNA had no effect on the reaction. These results suggest that part of the synthesised product is complementary to globin mRNA.

The unspecific RNA, synthesis of which increases with incubation time, is not identical with 6S RNA as judged from its sensitivity to RNase and from its S value of less than 4. The nature of this RNA was not investigated further, but it can easily be separated from the globin-specific RNA by chromatography on a cellulose column<sup>8</sup>. If an SDS-treated and phenolised reaction product is applied to such a column in the presence of 15% ethanol, the unspecific RNA does not absorb,

and the globin-specific RNA can be eluted only with buffer containing no ethanol. This latter RNA is RNase resistant and completely complementary to globin mRNA. It corresponds in quantity to the synthesised globin-specific RNA which was analysed directly in the enzymatic reaction product by renaturation. Therefore, cellulose column chromatography of the products of all primer-dependent reactions of  $\text{Q}_\beta$  replicase with heterologous RNAs as template, is recommended before further analysis.

The double-stranded material eluted from the cellulose column had an S value of about 7.5, as analysed by a sucrose gradient centrifugation in the presence of  $^{32}\text{P}$ -labelled *E. coli* RNA as internal marker (Fig. 2a). After denaturation the S value of most of the material shifted to 5.6 with some RNA sedimenting as a shoulder at 10S (Fig. 2b). The relatively small size of most of the single-stranded RNA product seems to result from premature termination of synthesis on the template RNA, thus leading to a double-stranded structure with a single-stranded tail. This argument is favoured by the comparatively large S value of the double-stranded structure which gets smaller on RNase treatment specific for single-stranded RNA (data not shown). The reason for the unfinished synthesis in the case of rabbit globin RNA as template is unknown but it should be noted that in the  $\text{Mn}^{2+}$ -type reaction of  $\text{Q}_\beta$  replicase with brome mosaic virus RNA as template, a smaller product of distinct size appeared in addition to the larger product<sup>1</sup>.

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## Naturally occurring antibody against unusually high serum DNA polymerase activity

We have reported unusually high DNA polymerase activity in sera obtained from chimpanzees infected with the hepatitis B virus<sup>1</sup>, and Kaplan *et al.*<sup>2</sup> reported hepatitis B-specific DNA polymerase activity in sera of human patients during post-transfusion hepatitis. We and others<sup>3–5</sup> also found DNA polymerase activities associated with Dane particles, the putative hepatitis B virus, or their naked core components. Reports<sup>6,7</sup> of the specific association of an e antigen–e antibody system with carriage of HB<sub>Ag</sub> prompted us to examine HB<sub>Ag</sub> positive sera containing unusually high levels of DNA polymerase activity for the presence of e antigen or e antibody. Subsequently, we studied the effects of sera containing e antibody on high DNA polymerase activity in sera from individuals with acute hepatitis B, chronic asymptomatic hepatitis B, chronic aggressive hepatitis B or chronic non-B hepatitis.

We have screened several hundred sera for high DNA polymerase activity using a standard radioisotope method<sup>8</sup>. Sera from normal healthy controls were found to contain low levels of polymerase activity, usually about 400 c.p.m.

Table 1 Effects of antisera on high serum DNA polymerase activity

Test serum No.	Patient serum No.	Serum description	Control activity (c.p.m.)	Adsorptions: c.p.m. % effect against control activity		
				anti-e	Adsorbed anti-e	anti-HB <sub>s</sub>
1	1	HB <sub>s</sub> Ag neg.	11,391†	6,706* /41% ↓	NT	NT
2	2	HB <sub>s</sub> Ag neg.	33,311†	17,689‡ /47% ↓	35,076§ /5% ↑	44,979¶ /35% ↑
3	3	HB <sub>s</sub> Ag neg.	19,564†	9,410* /52% ↓	NT	NT
4	4	HB <sub>s</sub> Ag neg.	18,713†	NT	NT	16,000   /14% ↓
5	5	HB <sub>s</sub> Ag neg.	95,432†	60,639‡ /36% ↓	99,978§ /5% ↑	91,644¶ /4% ↓
6	1:10 5§§	HB <sub>s</sub> Ag neg.	11,412†	6,053‡ /47% ↓	9,672§ /15% ↓	11,223¶ /—
7	6	HB <sub>s</sub> Ag neg.	4,229†	1,243* /71% ↓	NT	NT
8	7	HB <sub>s</sub> Ag neg.	10,453†	3,856‡ /73% ↓	NT	NT
9	8	HB <sub>s</sub> Ag pos. e neg.	881†	520* /36% ↓	NT	NT
10	9	HB <sub>s</sub> Ag pos. e neg.	100,981†	88,625* /14% ↓	NT	104,270¶ /4% ↑
11	9	HB <sub>s</sub> Ag pos. e neg.	100,981†	79,632‡ /22% ↓	NT	NT
12	10	HB <sub>s</sub> Ag pos. e neg.	125,135†	NT	121,437** /3% ↓	NT
13	11	HB <sub>s</sub> Ag pos. e Ag pos.	11,995†	NT	7,182,§ /40% ↓	NT
14	11	HB <sub>s</sub> Ag pos. e Ag pos.	9,304†	3,157* /70% ↓	NT	NT
15	11	HB <sub>s</sub> Ag pos. e Ag pos.	9,304†	5,358†† /51% ↓	NT	NT
16	12	HB <sub>s</sub> Ag pos. e Ag pos.	7,128†	3,493* /50% ↓	NT	6,731¶ /6% ↓
17	13	HB <sub>s</sub> Ag pos. e Ag pos.	4,125†	2,352‡ /43% ↓	NT	NT
18	14	HB <sub>s</sub> Ag pos. anti-e pos.	57,847†	24,205‡ /58% ↓	NT	NT
19	15	HB <sub>s</sub> Ag pos. anti-e pos.	34,614†	NT	NT	42,167   /22% ↑
20	15	HB <sub>s</sub> Ag pos. anti-e pos.	33,012†	11,634* /65% ↓	NT	NT
21	15	HB <sub>s</sub> Ag pos. anti-e pos.	26,257†	4,144‡ /84% ↓	NT	19,706¶ /25% ↓
22	16	HB <sub>s</sub> Ag pos. anti-e pos.	17,103‡‡	6,144‡ /66% ↓	NT	18,473¶ /8% ↑
23	16	HB <sub>s</sub> Ag pos. anti-e pos.	17,103†	6,430†† /64% ↓	NT	NT
24	17	HB <sub>s</sub> Ag pos. anti-e pos.	8,724†	NT	5,928** /32% ↓	NT
25	1:3 17¶¶	HB <sub>s</sub> Ag pos. anti-e pos.	5,350‡‡	NT	4,171§ /22% ↓	NT
26	18	HB <sub>s</sub> Ag pos. anti-e pos.	874†	693‡ /21% ↓	NT	NT
27	19	HB <sub>s</sub> Ag pos. anti-e pos.	36,872†	20,044* /46% ↓	NT	37,745   /2% ↑

DNA polymerase activity in serum was determined by a radioisotope method that measures the incorporation of <sup>3</sup>H-dTTP into an acid-insoluble product. The polymerase assay contained in a volume of 0.370 ml: 50 µl of serum and 260 µl 0.01 M Tris buffer, pH 7.8, containing 30 mM MgCl<sub>2</sub>·6H<sub>2</sub>O, 60 mM KCl, and 1.5% Triton X-100; the balance of the reaction mixture contained (final assay concentrations) mercapto-ethanol (2.5 mM), dATP, dCTP, dGTP (0.25 mM each), 50 µg carrier chicken cell DNA and <sup>3</sup>H-dTTP (0.90 µM, specific activity 54 Ci mmol<sup>-1</sup>). All reaction mixtures were incubated for 3 h at 37 °C; two 100-µl samples per assay were pipetted on to Whatman 3MM filter paper disks, which were then transferred to a solution of ice-cold 5% trichloroacetic acid (TCA) plus 2% pyrophosphate. The disks were washed four times with this solution, once with a 5% TCA solution, twice with 95% ethanol and then air dried. Each disk was counted in 10 ml of a toluene PPO (PPO = 2,5-diphenyloxazole)-POPOP (POPOP = 1,4-di-[2-(5-phenyloxazolyl)]-benzene) cocktail. Zero-time (T = 0 min) c.p.m. were typically 100 and were subtracted from the gross sample c.p.m. to yield the tabulated c.p.m. HB<sub>s</sub>Ag and anti-HB<sub>s</sub> content of sera were determined by solid phase radioimmunoassay techniques, using commercial reagents (Abbott). Measurement of e antigen and antibody was performed using rheophoresis plates (Abbott) with incubation at 27 °C in humidified Petri dishes. The standard e antigen and e antibody reagents used were obtained from HB<sub>s</sub>Ag positive blood donors. All adsorptions were carried out at 37 °C for 1 h, followed by overnight incubation at 5 °C (16 h). Test sera (50 µl each) were adsorbed with 50 µl normal human serum and centrifuged the following day at 5,000g for 30 min to pellet out immune aggregates; the supernatants were used as the control sera in these experiments. Adsorptions of test sera with anti-e and anti-HB<sub>s</sub> antisera were carried out in a similar manner. Anti-e antiserum (100 µl) was preadsorbed with 10 µl of either goat anti-human immunoglobulin A, G and M antiserum (or with horse antihuman IgG antiserum) before use as an adsorbant of the test serum. Conditions for preadsorption of the anti-e antiserum were as described above, except that overnight incubation was omitted. Preadsorption of normal human serum with either goat or horse antiserum before use as an adsorbant of test sera did not alter control sera DNA polymerase activity. NT, not tested.

\*Anti-e antibody positive serum No. 1 (human).

†Normal human serum No. 1 (NHS).

‡Anti-e antibody positive serum No. 2 (human).

§Goat anti-human immunoglobulin A, G and M antiserum.

¶ Anti-HB<sub>s</sub> antiserum (human).

|| Anti-HB<sub>s</sub> antiserum (chimpanzee).

\*\*Horse anti-human IgG antiserum (H and L chain specific).

††Anti-e antibody positive serum No. 3 (human).

‡‡Normal human serum No. 2.

§§1:10 dilution of test serum No. 5 in normal human serum.

¶¶1:3 dilution of test serum No. 17 in normal human serum.

||||Patient sera Nos 14, 15, 16, 17 and 18 represent serial bleedings of a single patient.



above background, whereas sera from acute or chronic cases of hepatitis B ranged in activity from 450 to 1,500 c.p.m. Several sera, however, had unusually high levels of DNA polymerase, ranging in activity from 4,000 to 173,000 c.p.m. All the sera used in our study were selected initially on the basis of very high DNA polymerase activity, without previous regard to the presence of e antigen or e antibody (Table 1). With these sera we found no correlation between this high DNA polymerase activity and the presence or absence of HB<sub>s</sub>Ag, e antigen or e antibody. It is interesting that high DNA polymerase activity was found in sera from individuals with chronic non-B hepatitis as well as in two individuals who had e antibody.

The inhibitory effect of serum containing e antibody on the DNA polymerase activity found in the above sera was unexpected. This inhibitory effect was not confined to serum samples from hepatitis B patients, since high DNA polymerase activity in sera from chronic non-B hepatitis patients was similarly suppressed by addition of e antiserum (for example, serum containing e antibody). Briefly, sera containing high DNA polymerase activity were mixed with either normal human serum (the control) or human serum containing e antibody or anti-HB<sub>s</sub> and incubated for 1 h at 37 °C and overnight at 5 °C; the mixtures were then centrifuged at 5,000g for 30 min. The supernatants were assayed for residual DNA polymerase activity. Substantial reductions in polymerase activity (up to 84%) were found in serum samples incubated with e antiserum in contrast to samples reacted with normal human serum or anti-HB<sub>s</sub>. For example, when two serum samples with control DNA polymerase activities of 9,300 and 26,500 c.p.m. were adsorbed with e antiserum, their polymerase activities were reduced to 3,150 and 4,140 c.p.m. respectively. This phenomenon was confirmed by repeating the assays using two other sera containing e antibody for the pre-incubation step. Sera containing anti-HB<sub>s</sub> antibody did not affect substantially any of the high DNA polymerase activities, indicating that the activity was not associated with HB<sub>s</sub>Ag. These findings strongly suggested the presence of a specific factor in serum containing e antibody that can inhibit DNA polymerase activity. Adsorption of e antiserum with goat anti-human IgG antiserum inactivated this factor, indicating that it was an antibody.

Statistical analysis of the effects of e antiserum, adsorbed e antiserum, and anti-HB<sub>s</sub> antiserum on test serum DNA polymerase activity was performed by first ranking the percentage effects (calculated as percentage depression or increase over control activity) of the antisera on the test serum activity and then inspecting the resulting array for possible separation points. Such a point occurred at 35% depression. Seventeen of the twenty-one test sera contained DNA polymerase activity which could be depressed by 36–84%. Only one of seven test sera examined contained DNA polymerase activity which could be depressed more than 36% (40%) by the e antiserum which had been pre-adsorbed with goat anti-human IgG. None of the ten anti-HB<sub>s</sub> adsorbed test sera had depressions (or increases) in polymerase activity  $\geq 36\%$  of the matching control activity. Student's *t* test was applied to the difference between proportions exceeding 35% depression (or increase) in activity to determine the statistical significance of the findings. For the e antiserum proportion (17/21) compared with adsorbed e antiserum proportion (1/7), the probability of e antiserum producing an excess of depressions on the basis of chance alone is  $P \leq 0.001$  ( $t_{26}=3.85$ ), strongly suggesting the involvement of an antibody (IgG) in the suppression of DNA polymerase activity. Similarly, the probability of producing an excess of depressions compared with anti-HB<sub>s</sub> (0/10) is  $P < 0.001$  ( $t_{29}=6.31$ ), indicating the absence (in the two different anti-HB<sub>s</sub> antisera used) of a naturally occurring antibody against DNA polymerase activity in the test sera. The significance of these findings was basically unchanged

when the effects of the antisera on high polymerase activity were analysed statistically using results from individual patient's sera instead of test sera. In this case, 14 of 16 patients' sera contained DNA polymerase activity which could be depressed 36–84% by adsorption with e antiserum.

As far as we know, this is the first report of a naturally occurring antibody which specifically inhibits high DNA polymerase activity in human serum. In addition, the inhibitory effect of e antiserum was not confined to DNA polymerase activity associated with HB<sub>s</sub>Ag positive sera, suggesting a more general role of the inhibitory factor (now believed to be an antibody) in suppressing unusually high DNA polymerase activity. These data do not show whether or not the inhibitory effect of e antiserum directly involves the DNA polymerase enzyme or its template. Furthermore, the polymerase activities reported here have not been characterised as specific to either host or virus; however, it is tempting to speculate on the existence of naturally acquired (occurring) anti-viral DNA polymerase antibodies which can either abort or modify the course of a viral infection or virus-induced disease.

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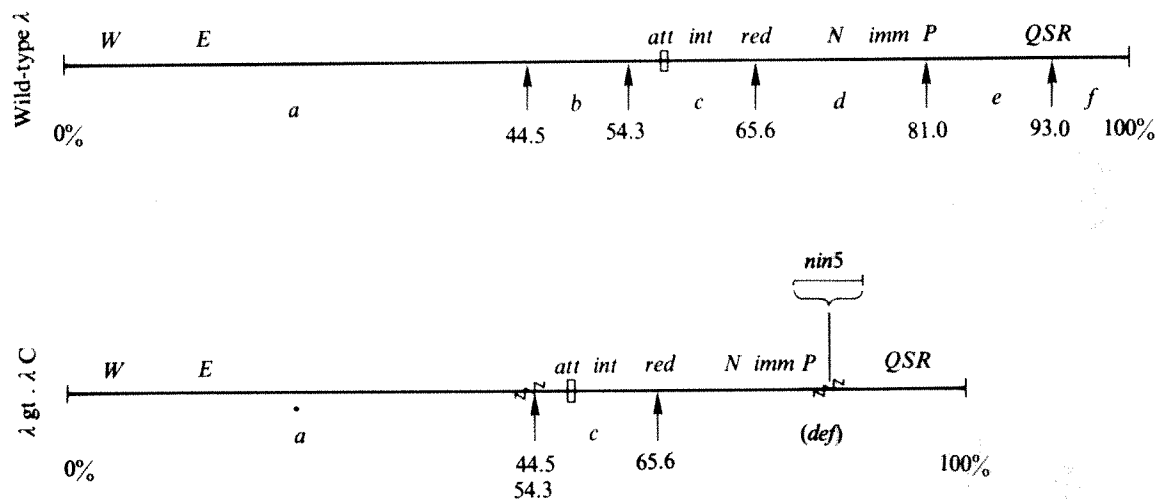
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## Safer derivatives of bacteriophage $\lambda$ gt $\lambda$ C for use in cloning of recombinant DNA molecules

THOMAS *et al.*<sup>1</sup> have constructed a mutant strain of bacteriophage  $\lambda$  ( $\lambda$ gt $\lambda$ C) which is especially well suited to studies involving the cloning of recombinant DNA molecules. Specifically, this bacteriophage, deleted of 15% of its genome, has only two *EcoRI* restriction endonuclease sites which, when cleaved, reduce the phage chromosome to three DNA fragments. The larger of these, the right- and left-hand arms of the chromosome, encode all functions necessary for lytic infection. The smaller, central fragment consisting of approximately 5,000 base pairs encodes the  $\lambda$  attachment site, the *Int* and *Xis* functions and a necessary part of the  $\lambda$ -generalised recombination system, but no genes necessary for phage propagation. Although left- and right-hand restriction fragments have all necessary  $\lambda$  genes, they seem to lack sufficient length of DNA for packaging into a viable phage particle<sup>1</sup>. This provides a powerful positive selection for recombinant DNA molecules consisting of isolated and rejoined right and left restriction fragments



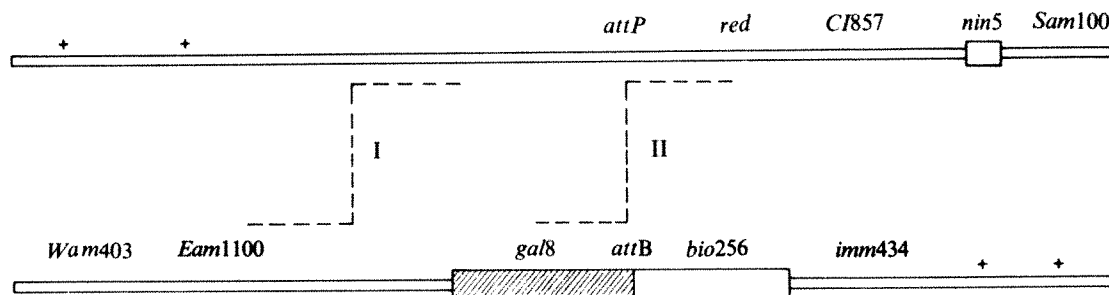
**Fig. 1** Characteristics of wild-type  $\lambda$  and  $\lambda$ gt- $\lambda$ C. *Eco*RI target sites given below line as %  $\lambda$  unit length<sup>12</sup>. *Eco*RI fragments are lettered *a* to *f* below line,  $\lambda$  genes above line. For  $\lambda$ gt- $\lambda$ C note that *b* fragment is removed and a hybrid RI target at 54.3% is regenerated<sup>1</sup>. Amber mutations in gene *S* were isolated by modifying the procedure of Goldberg and Howe<sup>7</sup> as follows.  $\lambda$ gt- $\lambda$ C lysogens of *E. coli* mutD<sup>3</sup> were obtained by isolating colonies from the centre of a phage plaque.  $\lambda$ -resistant clones of these lysogens were made by plating on EMBO plates seeded with *λvir* (ref. 8); 10-ml cultures of these  $\lambda$ -resistant,  $\lambda$ gt- $\lambda$ C lysogens were grown at 32 °C in tryptone broth to about  $2 \times 10^8$  cells ml<sup>-1</sup>. Cultures were induced by growing at 42 °C for 20 min and then at 37 °C for 3 h. Lysis was virtually complete by 60 min. After 3 h at 37 °C, culture was centrifuged for 10 min at 4,000 r.p.m. in a Sorval GLC-2. Pellet containing unlysed cells and debris was washed once with tryptone broth and centrifuged again. Pellet was resuspended in 0.5 ml tryptone broth containing enough  $\lambda$  antiserum to give a *K* value of 1. This mixture was incubated at 32 °C for 5 min and then diluted 100-fold in 0.01 M Tris, 0.01 M MgSO<sub>4</sub>, 0.1% gelatin (TMG). After centrifugation at 4,000 r.p.m. for 15 min, the pellet was washed with 45 ml TMG and recentrifuged. This pellet was resuspended in 1 ml TMG and incubated with several drops of chloroform at 37 °C for 5 min. The chloroform-treated mixture was plated on a mixed indicator of *supO* (N99) and *supF* (*Ymel*) bacteria at 37 °C. Turbid plaques, indicating growth on one indicator but not the other, appeared at a frequency of  $10^{-3}$ – $10^{-4}$ . Two isolates were characterised by growth on suppressor strains and by complementation as described by Shimada *et al.*<sup>8</sup>. Both mutants formed plaques on *supF* bacteria but plated at less than  $10^{-6}$  efficiency on *supO* hosts. In addition, both mutants plated well on *supC* (CA169) but grew poorly on *supE* (C600). Both isolates complemented all tested  $\lambda$  mutants except *Sam7*. The mutants were therefore designated *Sam100* and *Sam101*.  $\lambda$ Sam100 lysogens of *supO* hosts do not lyse after induction until chloroform is added.  $\lambda$ Sam101 lysogens of *supE* hosts behave similarly but 2 h after induction, some spontaneous lysis can be observed. Optimal phage yields (about 200–500 phage per induced cell) from induced *supE* lysogens are obtained by collecting phage 2 h after induction.

which have also incorporated a fragment of foreign DNA restoring the  $\lambda$  chromosome to viable size.

Although the  $\lambda$ gt- $\lambda$ C-*Escherichia coli* K12 host-vector system offers rather special advantages as a system in which to clone DNA fragments that might contain potentially biohazardous segments, the need for further modification to make this and other vectors even safer was made clear during the Asilomar Conference on Recombinant DNA Molecules<sup>2</sup>. Accordingly, we have introduced three muta-

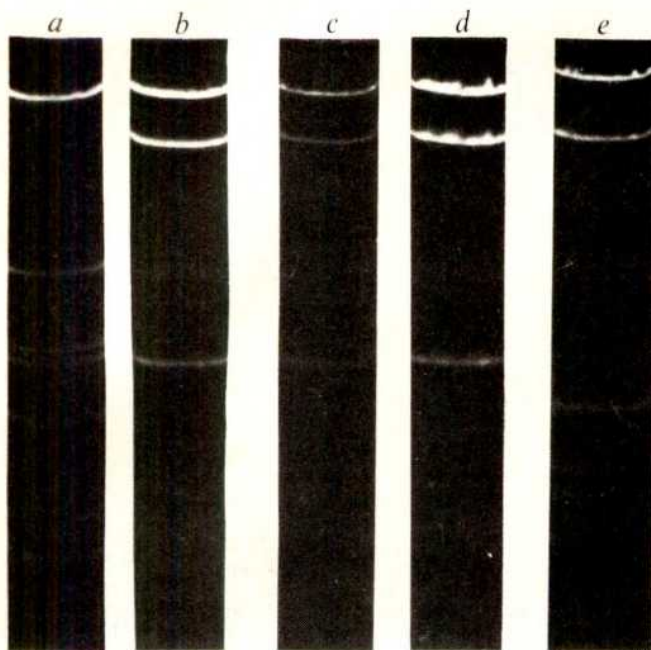
tions into the original  $\lambda$ gt- $\lambda$ C phage which reduce the possibility of its encountering a host in nature by at least a factor of  $10^6$  and which make it especially amenable to high titre growth in small and therefore more manageable volumes of medium. These modifications should make this phage suitable for use in experiments which require moderate biological containment barriers<sup>2</sup>.

Here we describe the construction of derivatives of  $\lambda$ gt- $\lambda$ C containing three amber mutations, *Sam100* *Eam1100* and



**Fig. 2** Construction of  $\lambda$ gt- $\lambda$ C *Wam403 Eam1100 Sam100*. Phage parent I (*a*) was  $\lambda$ gt- $\lambda$ C *Sam100* obtained as described previously. Phage parent II (*b*) (obtained as G96 from NIH stock collection) was  $\lambda$  *Wam403 Eam1100 gal8 bio256 imm434 cts1*. Strain *Ymel* was infected with both parents at a multiplicity of five of each phage. After 15 min adsorption at 32 °C, the infected cells were treated with ultraviolet light at a dose of 300 erg mm<sup>-2</sup>, diluted 1,000-fold in tryptone broth containing  $10^{-2}$  M MgSO<sub>4</sub>, and incubated at 40 °C for 70 min. Chloroform was added to complete lysis. Incubation at 40 °C insured a low level of *Int*-promoted recombination<sup>9</sup>. Dilutions of the lysate were plated on *Ymel recA56*. Parent I, but not parent II, will make plaques on this host because *bio256* deletes the *red* and *gam* genes<sup>10</sup>. These plaques were tested on *Ymel groE*<sup>11</sup>. Only parent II and recombinants carrying the *Eam1100* mutation will plate on this host. The presence of the *Wam403* mutation does not influence this test. Several isolates that made plaques on *Ymel recA56* and on *Ymel groE* were tested for their immunity by ability to grow on  $\lambda$  and phage 434 lysogens of *Ymel*. Because both *Eam1100* and *Wam403* are suppressed well on *supE* hosts but *Sam100* is not, the presence of *Sam100* was inferred by poor growth on C600. The presence of the *gal8* substitution was determined by measuring transduction to *gal*<sup>+</sup> of a *Ymel gal* deletion (SA825). Stocks with  $\lambda$  immunity also made clear plaques above 37 °C and turbid plaques at 32 °C indicating the presence of the *CI857* mutation. Complementation tests<sup>8</sup> were carried out to confirm that the phage contained *E*, *W* and *S* mutations. Finally, the presence of *nin5* was determined by plating on *E. coli* 'super nus' (M. Baumann and D. I. Friedman; N. S., unpublished). Two isolates were chosen for further study. One transduced SA825 to *gal*<sup>+</sup> and the other did not. Both were immunity  $\lambda$ , *E*<sup>-</sup>, *W*<sup>-</sup> and *S*<sup>-</sup>. The *gal*<sup>-</sup> recombinant presumably resulted from an event depicted by crossover I, the *gal*<sup>+</sup> recombinant from crossover II.





**Fig. 3** Electrophoretic separation of *Eco*RI fragments of DNA from wild-type  $\lambda$  and the  $\lambda$ gt- $\lambda$ C derivatives. *a*,  $\lambda$ c1857*Sam*7; *b*,  $\lambda$ gt- $\lambda$ C; *c*,  $\lambda$ gt- $\lambda$ C *Sam*100; *d*,  $\lambda$ gt- $\lambda$ C *Wam*403 *Eam*1100 *Sam*100; *e*,  $\lambda$ gt- $\lambda$ C *Wam*403 *Eam*1100 *Sam*100 *gal*8. Phage were prepared from induced lysogens or plate stocks<sup>5</sup>. Phage suspensions of  $1-20 \times 10^{10}$  phage ml<sup>-1</sup> were adjusted to a density of 1.35 g cm<sup>-3</sup> by addition of 0.5 g CsCl per 1.0 ml suspension and concentrated by centrifugation on a layer of CsCl at 1.70 g cm<sup>-3</sup> in 20 mM Tris-HCl, pH 7.8, and 10 mM MgCl<sub>2</sub> in an SW41 rotor for 1.0 h at 30,000 r.p.m. at 4 °C. The interface bands were pooled and banded to equilibrium by centrifugation in an SW56 rotor for 18 h at 35,000 r.p.m. at 4 °C. The suspensions were stored in CsCl at 0–5 °C. Phage suspensions ( $1-50 \times 10^{11}$  phage ml<sup>-1</sup>) in CsCl were brought to 1.0 mg ml<sup>-1</sup> Pronase (Calbiochem, nuclease-free) and dialysed at 37 °C for 90 min against 100 volumes buffer A (20 mM Tris-HCl, pH 7.8; 1.0 mM EDTA, 0.1 M NaCl) plus 0.002% Triton X-100. After gentle extraction with an equal volume of buffer A-saturated phenol, DNA was dialysed for 2–3 h against 100 volumes buffer A plus 0.002% Triton X-100, and then overnight against 100 volumes buffer A. The resulting DNA (0.010–1.2 mg ml<sup>-1</sup>) is stored at 0–5 °C. *Eco*RI was purified from the strain *E. coli* BRY 13.3/1100.5 (described elsewhere). The standard *Eco*RI reaction contained 0.1 M Tris-HCl, pH 7.9, 50 mM NaCl, 0.1 mM EDTA, 12 mM MgCl<sub>2</sub>, 10–50  $\mu$ g ml<sup>-1</sup> DNA, and enough *Eco*RI to completely digest 30–150  $\mu$ g  $\lambda$  DNA. The reaction was incubated for 30 min at 37 °C and stopped by addition of EDTA to 25 mM. After incubation for 5 min at 75 °C, the reactions were electrophoresed through 0.7% Agarose slab gels as described previously<sup>6</sup>. The DNA was stained by immersion of the gel in 0.0025% ethidium bromide for 3 min and visualised under an ultraviolet lamp.

*Wam*403. The *S* mutation, which prevents host lysis, was isolated by mutagenesis because the location of an RI cleavage site very close to or within the *S* gene makes it difficult to cross an *Sam* mutation into  $\lambda$ gt- $\lambda$ C without introducing unwanted RI sites (Fig. 1).  $\lambda$ Sam100 is not efficiently suppressed in conditions that suppress both *E* and *W* amber mutations. This produces an additional advantage in that one can obtain large phage concentrations in small, easily manipulated volumes of media. *Eam*1100 and *Wam*403 were crossed into  $\lambda$ gt- $\lambda$ C. The *E* gene product is the major head protein of the phage. The *W* gene product enables addition of tails to heads. Thus,  $\lambda$  carrying *Eam*1100 cannot make phage heads, whereas  $\lambda$  carrying *Wam*403 in the presence of a functional *E* gene, makes heads and tails but does not assemble these components. An additional effect of the *Eam*1100 mutation is that oligomeric  $\lambda$  DNA, the product of intracellular phage replication, is not cut into monomeric DNA with normal cohesive ends. We have tested the reversion frequency of each of these amber

mutations separately and found them to be of the order of  $10^{-6}$ . The *W* and *E* mutations therefore have an expected joint reversion frequency to *am*<sup>+</sup> of approximately  $10^{-12}$ . The probability of a host amber suppressor mutation arising spontaneously is approximately  $10^{-7}$ – $10^{-10}$  (ref. 4).

Two phage derivatives obtained from the cross shown in Fig. 2 have been characterised genetically by complementation tests and biochemically by *Eco*RI fragment patterns. The first,  $\lambda$ gt- $\lambda$ C *Wam*403 *Eam*1100 *Sam*100, has the restriction pattern characteristic of the parent phage,  $\lambda$ gt- $\lambda$ C (Fig. 3). The second,  $\lambda$ gt- $\lambda$ C *Wam*403 *Eam*1100 *Sam*100 *gal*8, has a larger left-arm fragment following *Eco*RI restriction and a smaller central fragment. In a separate mixing experiment with  $\lambda$ c1857, this latter fragment's molecular weight was found to be  $3.2 \times 10^6$ . We conclude that there is a single RI cleavage site within the *gal*8 substitution.

The presence of the *gal*8 substitution in the second phage enables establishment of lysogeny by recombination with the host genome and facilitates recognition of such lysogens. Although this is not desirable in recombination experiments with an uncharacterised constellation of DNA sequences, it may prove useful in the handling of a homogeneous, well-characterised fragment.

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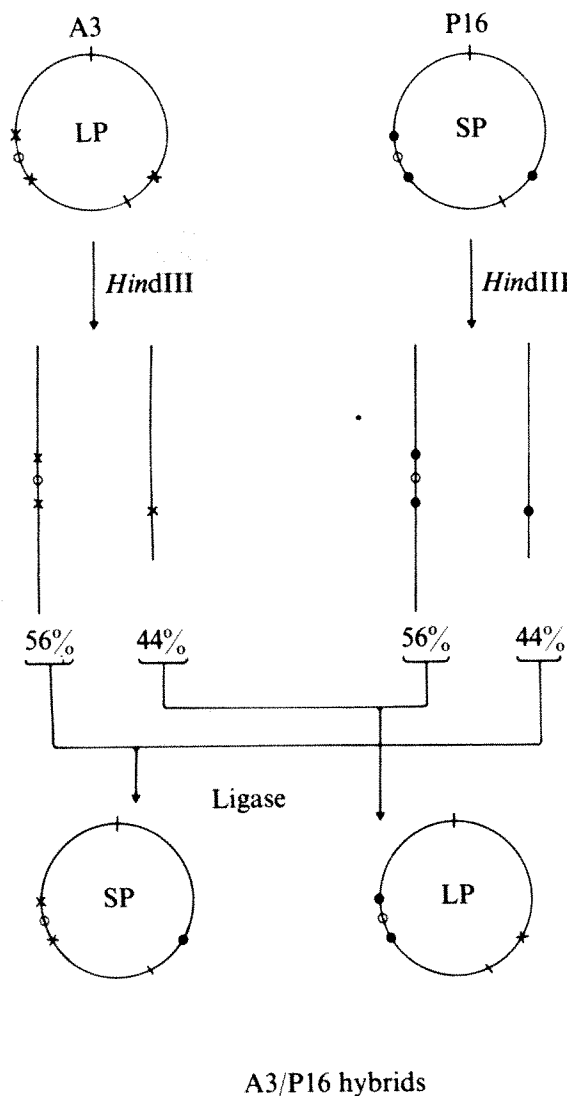
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## Construction of infectious polyoma hybrid genomes *in vitro*

WITH recent advances in nucleic acid biochemistry, it has become possible to recombine different animal viral DNAs at specific sites *in vitro*<sup>1,2</sup>. Specific hybrid viral genomes can be used in the genetic analysis of animal tumour viruses. With this aim in mind, we have made hybrids of two strains of polyoma (A-3 and P16). A-3 and P16 were developed from independent isolates and have been characterised both phenotypically and genotypically<sup>3,4</sup>. Using the hybrids it has been possible to begin to locate the DNA regions coding for phenotypic differences in the parent strains.

The two viruses differ phenotypically in their plaque morphology and haemagglutination (HA) properties; P16 produces small plaques and agglutinates guinea pig red blood cells at 20 °C as well as 4 °C whereas A-3 produces large plaques and agglutinates guinea pig red blood cells at 4 °C but not at 20 °C. The P16 and A-3 strains also differ genotypically. Three genotypic differences are readily dis-



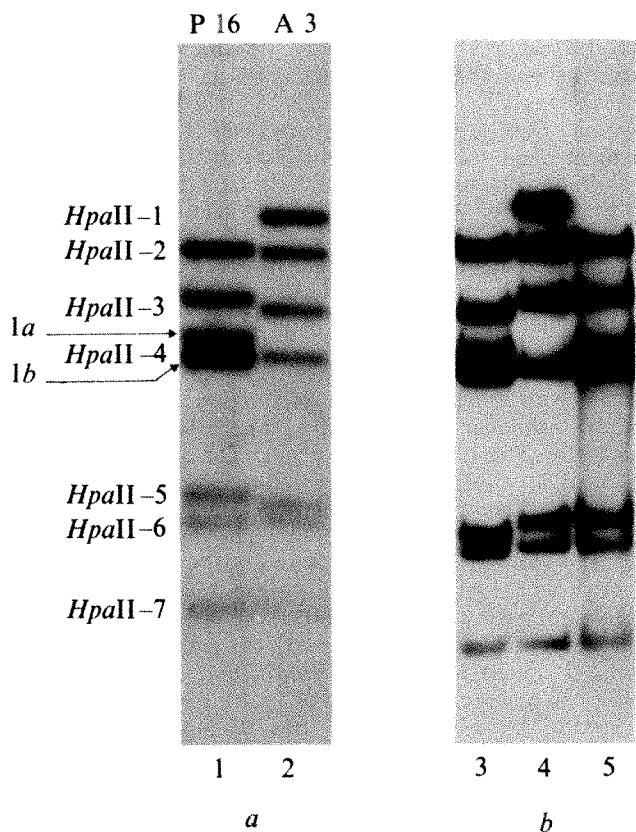


**Fig. 1** Construction of DNA hybrids *in vitro*. The A-3 and P16 strains of polyoma virus vary genotypically in at least three different sites denoted by (x) for A-3 and (o) for P16 on the circular genomes where (o) marks the origin and (-) marks the *HindIII* restriction endonuclease cleavage sites. The two strains also differ phenotypically; A-3 has a large plaque (LP) morphology whereas P16 has a small plaque (SP) morphology. Purified supercoiled (FI) DNA of A-3 and P16 viruses were digested with *HindIII* to yield two fragments approximately 44% and 56% of the genomes. The *HindIII* fragments from each viral DNA were separated by electrophoresis through 1.4% Agarose gels<sup>11</sup>, visualised by ethidium bromide staining<sup>11,12</sup>, individually sliced from the gels, eluted by gel electrophoresis and concentrated by ethanol precipitation. The A-3 *HindIII*-56% fragment was combined in a test tube with the P16 *HindIII*-44% fragment while the P16 *HindIII*-56% fragment was combined in another test tube with the A-3 *HindIII*-44% fragment. The fragments were covalently joined with *T*<sub>4</sub> DNA ligase in a reaction mixture containing 67 mM Tris (pH 7.6), 6.7 mM MgCl<sub>2</sub>, 0.67 mM dithiothreitol, 0.067 mM rATP, 300 µg per ml of each DNA fragment and  $2 \times 10^{-3}$  units *T*<sub>4</sub> DNA ligase by incubation at 5 °C for 16 h. Subsequent infection of mouse embryo cells with the hybrid DNAs for plaque assay facilitated the determination of the plaque morphology characteristics of the hybrid strains (LP and SP as indicated).

cerned in the *Haemophilus parainfluenzae* restriction endonuclease (*HpaII*) cutting patterns of their DNA (Fig. 2a and ref. 3). The most obvious difference between the P16 and A-3 *HpaII* cleavage patterns is that P16 is cleaved nine times by *HpaII* whereas A-3 is cleaved only eight times. The additional cleavage site of P16 is located within the largest *HpaII* fragment of A-3 (designated A-3 *HpaII*-1) and generates two new fragments (designated as P16 *HpaII*-1a and P16 *HpaII*-1b) which migrate in 3.3% polyacrylamide-0.5% Agarose gels on either side of the A-3

*HpaII*-fragment-4 (*HpaII*-4). In addition, the *HpaII*-3 and *HpaII*-5 fragments of A-3 are slightly smaller (migrate faster) than the equivalent P16 fragments (designated P16 *HpaII*-3 and P16 *HpaII*-5). Hybrids constructed from these two polyoma strains can therefore be studied both at the genotypic level and at the phenotypic level; hybrid formation can be confirmed by the *HpaII* cleavage patterns of the hybrid viral DNAs and the phenotypic differences of plaque size and HA ability can potentially be correlated with a specific region of the viral genome.

The construction of A-3 and P16 hybrid viruses is schematically shown in Fig. 1. Both A-3 and P16 are cleaved twice by the *HindIII* restriction endonuclease yielding two fragments of approximately 44% and 56% (designated *HindIII*-44% and *HindIII*-56% respectively) of the genome<sup>3</sup>. These fragments were purified by gel electrophoresis through 1.4% Agarose gels. The *HindIII*-44% of A-3 was combined with the *HindIII*-56% of P16 and, vice versa, the P16 *HindIII*-44% was combined with the A-3 *HindIII*-56%. To encourage intermolecular rather than



**Fig. 2** *a*, An autoradiograph showing the restriction nuclease cleavage patterns of (1) <sup>32</sup>P-labelled P16 DNA and (2) <sup>32</sup>P-labelled A-3 DNA (modified from Fried *et al.*<sup>3</sup>). The numbers at the side of the gel designate the nomenclature of the bands. The gels (20 × 20 cm) were composed of 3.3% acrylamide-0.17% bis-acrylamide-0.5% Agarose and run as described previously<sup>7</sup>. In P16, *HpaII*-1 is absent and two new fragments (1a and 1b) are present. The sizes of *HpaII*-3 and *HpaII*-5 can be seen to vary between the A-3 and P16 strains. *b*, Secondary whole mouse embryo cells (WME) were infected with each A-3/P16 hybrid DNA, overlaid with agar and stained with neutral red after 9 d incubation at 37 °C. Plaques of each hybrid were picked, virus stocks of each plaque were produced at low multiplicity<sup>10</sup> and <sup>32</sup>P-labelled form I DNA was prepared as previously described<sup>3,7</sup>. The labelled DNAs were digested with *HpaII* and the resulting fragments were electrophoresed through a 3.3% acrylamide-0.17% bis-acrylamide-0.5% Agarose slab gel. The autoradiograph shows the representative *HpaII* cutting pattern of (3) the small plaque hybrid P16 *HindIII*-44% + A-3 *HindIII*-56%, (4) the large plaque hybrid A-3 *HindIII*-44% + P16 *HindIII*-56%, and (5) small plaque P16 control. For both the hybrids, five plaques were picked and tested. Five out of five plaques in each case showed the expected cutting patterns. In the gels in *a* and *b* *HpaII*-8 has been run off the gel. There was no difference in the electrophoretic mobility of *HpaII*-8 between the A-3 and P16 strains or any of the plaque isolates.



Table 1 Analysis of the phenotypic properties of A-3/P16 hybrids

DNA	Plaque size*	HA at 20 °C†	HA at 4 °C
A-3 Form I DNA	LP	—	+
P16 Form I DNA	SP	+	+
A-3 <i>Hind</i> III-44% + P16 <i>Hind</i> III-56%	LP	—	+
P16 <i>Hind</i> III-44% + A-3 <i>Hind</i> III-56%	SP	+	+

\*LP, large plaque morphology; SP, small plaque morphology<sup>4</sup>.

†Ability of virus stocks derived from picked plaques to haemagglutinate guinea pig red blood cells at 20 °C.

(—) indicates less than 10 HA were detectable; (+) indicates HA titres between 160 and 1280. There was no difference in the HA titres of the P16 type at 4 and 20 °C.

intramolecular joining of fragments, each DNA was present at a concentration of 300 µg ml<sup>-1</sup>. The combined fragments were first heated to 50 °C to ensure complete denaturation of the four-base overlap at the restriction nuclease cutting site<sup>5</sup> and then cooled slowly to 5 °C and allowed to re-anneal at 5 °C for 30 min. The resulting hybrid DNAs were covalently closed by T<sub>4</sub> DNA ligase (a gift from Dr Ian Molineux). In the conditions used, approximately 90% of the DNA molecules were judged to have been ligated. This was determined by observing the disappearance of molecules with the electrophoretic mobility of the 44% and 56% fragments and the appearance of DNA with an electrophoretic mobility for molecules 88–112% the size of polyoma. Low levels of unligated *Hind*III-44% and 56% fragments and intramolecularly ligated *Hind*III-44% and 56% fragments were observed in gels but accounted for less than 10% of the ligation products. The remaining 90% of the DNA products migrated in 1.4% Agarose gels with a mobility equivalent to linear and circular forms of molecules 88%, 100% and 112% the length of polyoma DNA. Random intermolecular associations account for the size distribution of products.

Secondary whole mouse embryo cells (WME) were infected with the hybrid DNA products using the DEAE-dextran technique as described by Thorne *et al.*<sup>6</sup> for polyoma DNA. The results of the plaque assays with DNA are presented in Table 1. The *Hind*III-44% and *Hind*III-56% fragments alone had negligible contaminating infectivity (less than 1 × 10<sup>2</sup> PFU per µg DNA). The combined *Hind*III-44% and -56% fragments without ligation had a specific infectivity of 3 × 10<sup>3</sup> PFU per µg DNA; thus the cell is capable of ligating the two *Hind*III fragments at a low level. The *in vitro* ligated P16 *Hind*III-44%/A-3 *Hind*III-56% and A-3 *Hind*III-44%/P16 *Hind*III-56% ligated hybrid DNAs had a specific infectivity of 1 × 10<sup>3</sup> PFU per µg DNA. In control experiments, A-3 *Hind*III-44%/A-3 *Hind*III-56% ligated DNA also had a specific infectivity of 1 × 10<sup>3</sup> PFU per µg DNA indicating that the hybrid viral DNAs are as efficient in plaque formation as *in vitro* constructed parental DNA. Untreated supercoiled FI polyoma DNA or singly-nicked FII DNA had a specific infectivity of 1 × 10<sup>6</sup> PFU per µg DNA. Analysis of the plaque size produced by the A-3/P16 hybrid DNA molecules revealed that the plaque morphology depended on the source of the *Hind*III-44% fragment; the small plaque morphology of P16 was observed for the P16 *Hind*III-44%/A-3 *Hind*III-56% hybrid while the large plaque morphology of A-3 was observed for the A-3 *Hind*III-44%/P16 *Hind*III-56% hybrid. The haemagglutination characteristics also segregate with the *Hind*III-44% fragment (Table 1) in a manner similar to plaque morphology.

To confirm that the hybrid DNAs actually contained the genetic information of the two different parental DNAs, plaques of the hybrid viruses were picked and virus stocks produced. The viral DNA derived from <sup>32</sup>P-labelled infected cells was digested with *Hpa*II restriction endonuclease. Analysis of the fragments by gel electrophoresis (Fig. 2b) showed that the hybrid viruses constructed from the P16 and A-3 *Hind*III fragments exhibit *Hpa*II cutting patterns expected for such hybrids. From the physical order

of polyoma restriction nuclease fragments<sup>7</sup>, it is known that the *Hpa*II-3 and *Hpa*II-5 are located in the *Hind*III-56% fragments whereas the portion of the *Hpa*II-1 fragment containing the new *Hpa*II cutting site of P16 is located in the *Hind*III-44% fragment. The small plaque hybrid virus constructed from the P16 *Hind*III-44% and A-3 *Hind*III-56% fragments contains *Hpa*II-5 fragments which are smaller (migrate faster) than the equivalent P16 fragments and yet the hybrid contains the additional *Hpa*II cutting site characteristic of P16. The large plaque hybrid virus constructed from the A-3 *Hind*III-44% and P16 *Hind*III-56% fragments contain *Hpa*II-3 and *Hpa*II-5 fragments which comigrate with the corresponding P16 *Hpa*II fragments yet the hybrid does not contain the additional cutting site in *Hpa*II-1 fragment which is characteristic of the P16 type.

Thus, the *Hpa*II fragment patterns of the viruses confirm that hybrids between A-3 and P16 strains have been constructed and that they contain the sequences expected by our experimental approach. Furthermore, it is clear that the phenotypic difference between the P16 and A-3 strains of polyoma are not related to the genotypic differences located in the *Hind*III-56% fragment (that is the *Hpa*II-3 and *Hpa*II-5 fragment size differences). The genotypic difference which accounts for the phenotypic differences of the two strains must reside in the *Hind*III-44% fragment. Another plaque morphology variant and several late mutants have been mapped by a maker rescue technique in the *Hpa*II-1 sequences contained in the *Hind*III-44% fragment (L.K.M. and M.F., manuscript in preparation). This region also contains the additional *Hpa*II cutting site found in the P16 strain.

More exact determination of the locations of mutations affecting phenotype can be made by forming hybrid DNAs using different restriction nuclease fragments. Recently we have been able to construct hybrid molecules by ligation of restriction nuclease fragments produced by an enzyme from *H. haemolyticus* (*Hha*I). This enzyme produces fragments which contain a two-base overlap at their termini (Roberts and Myers, personal communication).

It has been possible to take advantage of the difference in restriction enzyme cleavage sites to analyse *in vivo* recombinants between adenovirus-2 ND<sub>1</sub> and adenovirus-5 (refs 8 and 9). We have used this type of genotypic analysis to characterise *in vitro* recombinants of polyoma. Construction of hybrid viruses *in vitro* provides a rapid and direct method for introducing genotypic markers into different virus strains.

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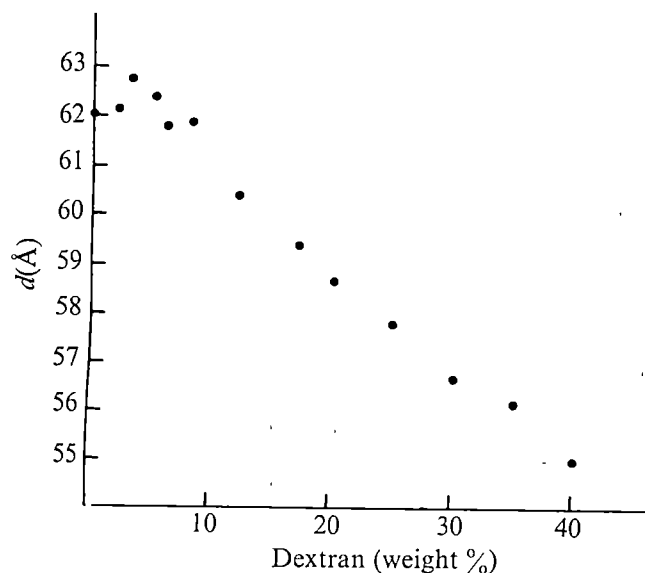
## Measurement of forces between lecithin bilayers

CELL fusion<sup>1</sup> and vesicle-membrane<sup>2</sup> or vesicle-synapse<sup>3</sup> fusion are currently thought to involve distance-dependent interactions between membranes. We have devised a general method for measuring such forces between planar phospholipid membranes, and report here results for the specific case of egg lecithin (phosphatidyl choline) bilayers in water.

When immersed in an excess amount of water, bilayers of zwitterionic lecithin form a lamellar lattice where the equilibrium bilayer separation is 27.5 Å (refs 4-7). We found that below this equilibrium spacing there is an apparent repulsion between bilayers, a repulsion that falls off exponentially with bilayer separation, with a decay distance of ~1.9 Å. The repulsive force for a separation < 22 Å is of the order of atmospheres, and reaches 10 atm by 17.5 Å. Our results indicate further that at the equilibrium spacing in pure water, repulsion is balanced by an attractive force between bilayers of ~6.5 × 10<sup>4</sup> dyne cm<sup>-2</sup>, or 0.065 atm.

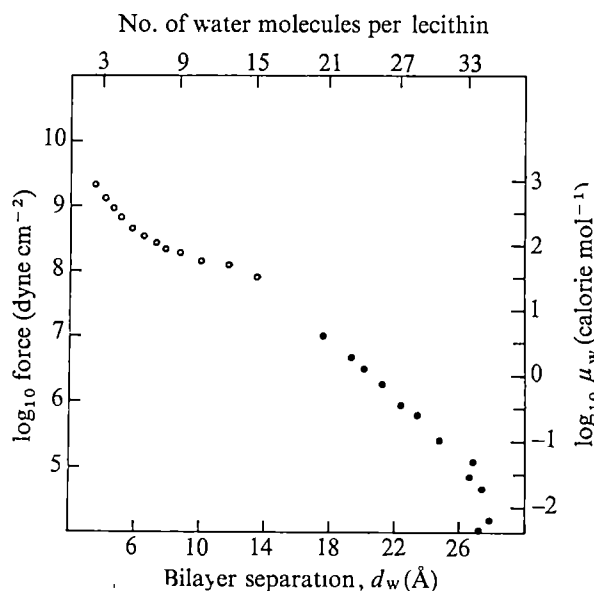
To measure the net repulsion or 'disjoining pressure'<sup>8</sup> we immerse pure egg lecithin into solutions of very high molecular weight dextran, rather than into pure water. The branched dextran molecules, having radii of gyration of the order of hundreds of Angstroms<sup>9,10</sup>, apparently cannot enter the well defined lamellar structure which still forms. (Enclosing the lipid in dialysis membranes before exposure to dextran solutions has no observable effect on the lamellar phase.) At each higher dextran concentration, the lamellar phase must come to

**Fig. 1** Lamellar repeat distance  $d$  as a function of dextran concentration. Chromatographically pure samples of egg lecithin (~30 mg) prepared by the method of Singleton *et al.*<sup>20</sup> were dispersed in 10 ml (10 g) of water or dextran (BDH: molecular weight 200,000-275,000) solution with the aid of sonication (30 s at half power on a Biosonic III). The lamellar lecithin phase was separated by ultracentrifugation. Scatter of points near 0% dextran is not systematic, but apparently arises from manipulation of the lipid. Repeat distances determined by X-ray diffraction are accurate to 0.1 Å.



equilibrium with water of lower chemical activity. Mechanically, the dextran solution exerts an osmotic pressure on the lamellar phase preventing full swelling of the lattice. At the new equilibrium, and for each dextran concentration, repulsion between bilayers is equal to the osmotic pressure of the dextran solution. The osmotic pressure is measured directly with a mercury manometer, or with pressure gauges. The resulting lattice spacings are measured by X-ray diffraction<sup>4-7</sup>. These spacings are shown in Fig. 1, where we plot the lattice repeat distance against dextran concentration.

To obtain a force-separation curve, we must calculate the spacing between leaflets at each dextran concentration. The total lattice repeat distance  $d$  is pictured as the sum of a biomolecular lipid layer of thickness  $d_l$  and a water layer of thickness  $d_w$ . To find  $d_l$  and  $d_w$  from  $d$  in Fig. 1, we use our X-ray diffraction data<sup>7</sup> for repeat spacings  $d$  in the one phase lamellar lattice formed in ~50% water. The state of the one-phase system is defined by mixing set amounts of water and lipid (of volume fraction  $\phi$ ). When  $\phi$  is set, one can say  $d_l = \phi d$  and  $d_w = (1 - \phi)d$ . The lamellar repeat  $d$  increases monotonically with added water (until the limit of swelling where the second, pure water, phase begins to form). The same set of limited-water states may be reached by removing water osmotically from the fully swelled system. Specifically, one is observing the same thermodynamic state for lattices of a given  $d$  whether the lipid-water ratio has been determined by gravimetric means or osmotically. Then the  $d$  spacings of Fig. 1 can be converted to  $d_w$  and  $d_l$  thicknesses as described in the table. The data are shown as a force against separation curve in Fig. 2.



**Fig. 2** Curve of force as a function of separation. Chemical potentials of water in the dextran solution (●) are found from  $\mu_w = \text{osmotic force} \times \text{specific volume of water}$  (using present data for the osmotic force). Those from Elworthy's vapour pressure (○) are derived from  $\mu_w = -RT \ln(p_w/p_w^{\text{sat}})$ , assuming water density is not noticeably changed, and are measured in calorie mol<sup>-1</sup>. Separation and water content per lecithin are as in Table 1. The relationship for pressure,  $P_0 \exp(-d_w/\lambda)$ , is convenient for discussing the osmotic data. Curve fitting to our osmotic pressure data (●) for 17.5 <  $d_w$  < 26.6 Å gives  $P_0 = 1.0 \times 10^{11}$  dyne cm<sup>-2</sup>,  $\lambda = 1.93$  Å.

The influence on  $d_l$  of any mono- or disaccharide breakdown products of dextran intercalated between bilayers is probably small. Besides the observed irrelevance of a dialysis membrane between dextran solution and lipid, we know that the replacement of water by glucose or sucrose solutions (of concentrations up to 40% by weight) has negligible effect on  $d$ ,  $d_l$  and  $d_w$  when used in the one-phase lamellar system<sup>7</sup>.

The lipid thickness  $d_l$  increases with decreasing water content (Table 1). This increase in thickness itself indicates the repulsion

Table 1 Analysis of osmotic stress data

Weight per cent dextran	Osmotic pressure	$d$	$d_1$	$\phi$	$d_w$	$\log_{10} F$ (dyne cm <sup>-2</sup> )	$\log_{10} \mu_w$ (calorie mol <sup>-1</sup> )	Water- lecithin mol ratio
40		55.0	37.7	0.69	17.3			20.1
39.3	739				(17.5)	6.994	+0.627	
35		56.2	37.2	0.66	19.0			22.4
32.5	351				(19.3)	6.671	+0.304	
30		56.7	37.1	0.65	19.6			23.2
28.5	243				(20.1)	6.511	+0.144	
25	141	57.8	36.6	0.636	21.2	6.268	-0.092	25.4
20	65.8	58.7	36.3	0.62	22.4	5.943	-0.42	27.1
17	43.8	59.4	36.0	0.61	23.4	5.766	-0.60	28.5
12	19.5	60.4	35.6	0.59	24.8	5.415	-0.95	30.6
8	9.1	61.9	35.1	0.57	26.8	5.082	-1.28	33.5
6	5.4	61.8	35.2	0.57	26.6	4.853	-1.51	33.2
5	3.6	62.4	35.0	0.56	27.4	4.681	-1.69	34.4
3	1.2	62.8	34.9	0.56	27.9	4.204	-2.16	35.1
2	0.8	62.2	35.0	0.56	27.2	4.028	-2.34	34.1
0	0	62.1	35.1	0.57	27.0			33.7

Osmotic pressures of the dextran solutions were made directly using a mercury manometer or pressure gauge. Solutions connected to the manometer were in contact with distilled water by way of a dialysis membrane. Putative volume fractions of lipid,  $\phi$ , in the lamellar phase were found by matching against separations seen in the single phase formed at low total water content<sup>7</sup>. Separation  $d_w$  is then  $(1-\phi)d$ , where the lamellar repeat distance  $d$  is measured by X-ray diffraction. Values in parentheses are interpolated between adjacent measured values. The definition of  $d$  as a separate layer containing only water is arbitrary. The aqueous region could also be defined to include the lipid polar moiety<sup>6</sup>. In that case the apparent thickness  $d$  will be greater than the values derived here. It is also a matter of convenience to picture the lipid-water interface as a sharp step where it is actually an interface of thickness comparable to the dimensions of the polar groups

between bilayers<sup>11</sup>, which is being quantitatively measured here.

At a separation of  $27.5 \pm 0.5$  Å, the lamellar lattice is in thermodynamic equilibrium with pure water. Because of its rapid exponential variation, the repulsive component apparently dominates the net interaction for separations only a fraction of an Angstrom less than the equilibrium separation in water. By taking the repulsion curve  $P_0 \exp(-d_w/1.9 \text{ Å})$  derived from fitting the experimental points of Fig. 2 that lie in the range  $17.5 < d_w < 26.6$  Å, and by extrapolating to the equilibrium separation of 27.5 Å in pure water, we estimate the repulsive part of the force between bilayers at equilibrium in water. Since this repulsion is equal and opposite to bilayer attraction, this method provides a first estimate of the interlamellar attraction force,  $5 \times 10^4$ – $8.5 \times 10^4$  dyne cm<sup>-2</sup>. The error, which appears among different lipid preparations, and probably results from handling the lipid, here assumes an ambiguity of 0.5 Å in the determination of the 27.5 Å equilibrium spacing.

Our results using osmotic stress on the lamellar lattice are supplemented by those of Elworthy<sup>12</sup> (supplied to us in tabular form) who determined the amount of water imbibed by egg lecithin as a function of aqueous vapour pressure,  $p_w$ . The chemical potential,  $\mu_w = -RT \ln(p_w/p_w^{\text{sat}})$ , measures the work of transferring water from the lamellar lattice vapour pressure  $p_w$  to bulk water (vapour pressure  $p_w^{\text{sat}}$ ). By dividing the work per mol by the molar volume (the value for pure bulk water is used here), the force per area between the bilayers from which water is removed is obtained. Lamellar repeat distances and bilayer separations are known from X-ray diffraction on lecithin<sup>7</sup> at these limited amounts of water. These vapour pressure measurements are plotted as the open circles in Fig. 2. (The strict similarity of the egg lecithin used in the imbibing and X-ray experiments is not established. The temperature in both studies was 25 °C, but was not strictly controlled in our case.)

The curve of force as a function of separation in Fig. 2 suggests that the vapour pressure and osmotic pressure experiments are consistent. But comparison is difficult because there is a 4 Å gap in data (between 13.5 and 17.5 Å separation). To this extent there does not seem to be a sharp jump in the work of removal of water at any point in the curve from 3 to 33 molecules of water per lecithin; within the limits of these measurements, there is a smooth change in the work of removing water near the lecithin polar groups. It is clear that the simple exponential law (solid points, osmotic data) is not maintained

when more than two-thirds of the original water is removed from the lamellar lattice.

Analyses of the amount of water near lecithins using thermal<sup>13</sup> and resonant<sup>14</sup> probes reveal 10–12 'hydrating' or 'bound' water molecules per lecithin molecule, with another 11 waters 'trapped' by each lecithin<sup>14</sup>. These probes would suggest that the remaining water is bound loosely enough to be considered bulk water. But the finite work of removal,  $\mu_w$ , which we measure osmotically shows this is not so.

The repulsion between lecithin bilayers probably arises both from electrostatic interaction between charged groups and from energy of hydration of all polar groups on the molecule. In the presence of solvent boundaries, as occur here between lipid and water, the change in hydration might be visualised in terms of electrostatic image forces. Between molecules without charged groups, repulsion would occur because of hydration energies alone. The exponential force measured here (Fig. 2) does not fit a model<sup>17</sup> where the lecithin polar group is thought to form an internal double layer.

The attractive force is of the order of magnitude expected for van der Waals' forces in lipid-water multilayers<sup>18</sup>. Its identity with these forces remains to be established by determination of the attractive force as a function of separation. This we are doing by applying osmotic stress to lamellae containing varied fractions of charged lipids in salt water media. We measure double-layer repulsions which will increase the equilibrium separation<sup>7,19</sup>. From each lamellar repulsion curve we may infer an attractive force at each equilibrium separation.

The work of pushing together two electrically neutral zwitterionic phospholipid bilayers can be significant compared with energies available from thermal motion in collision. To bring two faces of area  $100 \text{ Å}^2$  to even 15 Å separation would require about  $8 \times 10^{-13}$  erg or 20  $kT$  units. Alternatively, using the Derjaguin step approximation<sup>15,16</sup> to convert the interaction of two parallel planes to one between a planar bilayer and a spherical bilayer vesicle, a sphere of outermost radius 300 Å coming within 14 Å of contact also feels a barrier of about 20  $kT$ . Such large energies suggest that when fusion of two pure lipid vesicles is in fact observed experimentally, there might be some destabilisation or transient rearrangement of the phospholipid molecules to push apart the polar head groups. This would allow the hydrocarbon moieties to merge without requiring a large amount of work to remove water from the polar groups as observed here for uniform planar bilayers.

We hope that coupled energetic and structural measurements,

such as we report here, will lead to more precise analyses of the forces responsible for lamellar stability.

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## Stimulation and suppression of response of mouse T cells to the schistosomules of *Schistosoma mansoni* during infection

INFECTION of mice with cercariae of *Schistosoma mansoni* produces a disease with immunopathology similar to human bilharziasis<sup>1</sup>, the chronic hepatosplenomegaly being probably secondary to granulomatous reactions to eggs deposited in the tissues<sup>2</sup>. It has been shown that thymus-derived lymphocytes have a major role in the granulomatous reaction<sup>3,4</sup>, but the reaction of the T cells to earlier stages of the parasite has not hitherto been studied. We have investigated one such reaction—the helper T-cell response to the schistosomule—by using schistosomules as carriers for a hapten and measuring the anti-hapten response in normal and infected mice. In analogous experiments using protein carriers, the anti-hapten response has been shown to be due to cooperation between T cells and B cells<sup>5</sup>. We have found evidence for a specific T-dependent helper response which is greatly increased during the first 2 weeks after infection and subsequently diminishes, and we suggest that the diminution may partly represent a specific suppression of helper T-cell function.

(C57BL×BALB/c)F<sub>1</sub> mice and *nu/nu* (athymic) mice of various genotypes were infected with 40 cercariae of *S. mansoni* on the shaved abdomen<sup>6</sup>. Schistosomules were prepared *in vitro* by mechanical agitation to cercariae in a Vortex mixer<sup>7</sup>, isolation of the cercarial bodies by layering the mixture of bodies and tails on to the Hanks' buffered salts solution (HBSS) in a test tube, where the bodies sink to the bottom in 10 min, and incubation of this pellet for 24 h at 37 °C in RPMI-1640 with 20 mM HEPES in atmosphere of 5% CO<sub>2</sub> in air. The resulting schistosomule preparations were con-

**Table 1** Carrier effect of schistosomules in the antibody response of normal mice to TNP

Mice	No. of schistosomules injected intravenously	TNBS used for coating (mg/ml)	No. of mice	4 d anti-TNP PFC per spleen (geometric mean ± s.e.)
Normal	—	—	6	116(92–147)
	100	—	9	260(200–338)
	100	0.5	3	980(922–1,040)
	100	1.0	4	603(443–820)
	100	1.4	3	2,996(2,287–3,926)
	100	2.0	3	1,395(1,186–1,644)
	100	5.0	13	1,765(1,505–2,070)
	100	14.0	3	3,737(3,350–4,169)
	300	5.0	3	6,269(5,596–7,025)
	900	5.0	3	26,037(20,672–32,796)
	2500	5.0	7	31,466(22,403–44,196)
	2000	1.4	3	20,527(19,204–21,942)
Nude	2000	14.0	3	28,639(21,061–38,942)
	100	5.0	3	840(804–871)
	2000	5.0	5	4,755(3,750–6,748)

taminated with less than 5% cercarial tails. For vaccination, schistosomules were fixed in 0.06% formaldehyde for 5 min, followed by three washings in HBSS. For use as carriers for the hapten, schistosomules were coated with trinitrophenol (TNP) by a modification of the method of Rittenberg and Pratt<sup>8</sup>: 5,000–10,000 schistosomules were incubated in a 0.5% solution of trinitrobenzene-sulphonic acid (TNBS) in 0.28 M cacodylate buffer (pH 6.9), for 30 min, at 37 °C, then washed with modified barbital buffer (pH 7.1), followed by three washes in HBSS. TNP-coated schistosomules were injected into mice and the direct anti-TNP plaque forming cell (PFC) responses in their spleens were measured at various intervals thereafter, using lightly TNP-coated sheep erythrocytes<sup>9</sup>, by the slide method<sup>10</sup>. Preliminary experiments showed that intravenous injection produced a better response than intraperitoneal and that the response was maximal if assayed 4 d after infection. This procedure was adopted accordingly for experiments on groups of normal, nude, infected or vaccinated mice. For specificity controls, other groups of infected mice were injected intravenously with TNP-coated horse erythrocytes and the TNP response measured as above.

In normal mice, schistosomules showed a weak carrier effect, at least 900 TNP-schistosomules being needed to give a substantial anti-TNP response. The use of higher or lower TNBS concentrations for coating schistosomules did not greatly affect the result. Nude mice responded very much less well suggesting that the carrier effect was largely T-cell mediated (Table 1).

In mice infected with 40 cercariae, as little as 100 TNP-schistosomules produced a massive anti-TNP response, greatest when the TNP-schistosomules were injected 10 d after the

**Table 2** Failure of carrier effect to increase following a second infection with *S. mansoni* cercariae

	Anti-TNP PFCs per spleen (geometric mean ± s.e.)		
	0	7	10
First infection (40 cercariae)	1,765 (1,505–2,070)	88,495 (80,433–97,366)	233,675 (218,488–249,917)
Second infection at 5 weeks (40 cercariae)	25,098 (20,651–30,503)	20,973 (14,760–29,801)	38,193 (33,650–43,348)
Second infection at 10 weeks (40 cercariae)	4,971 (3,522–7,016)	5,783 (5,389–6,205)	11,181 (9,400–13,300)

(C57BL×BALB/c)F<sub>1</sub> mice were infected with 40 cercariae either once or twice at 5- or 10-week intervals, and injected with 100 TNP-schistosomules 7 or 10 d later.

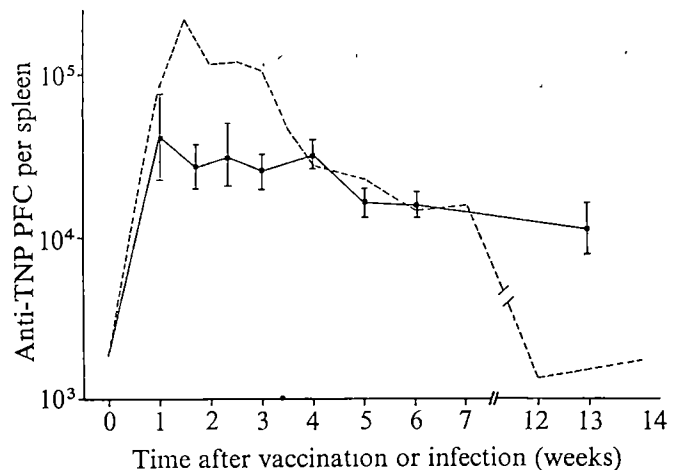
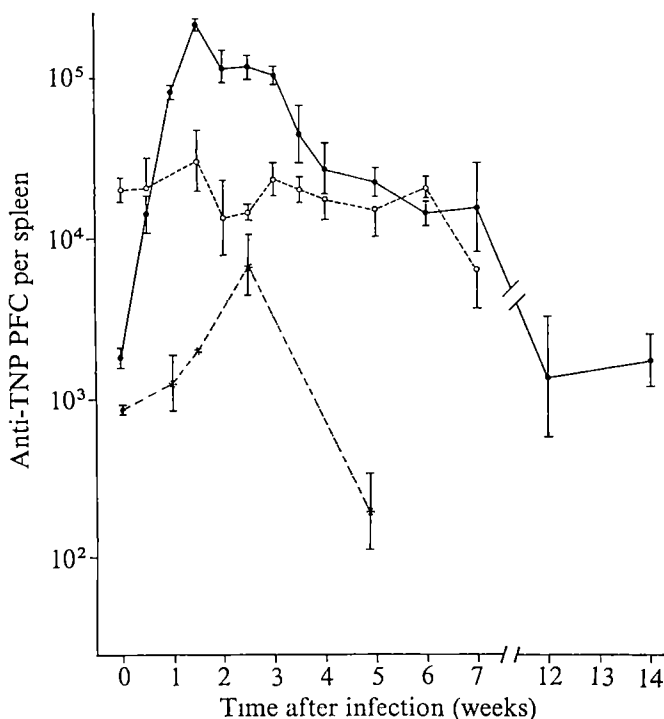


infection, and thereafter declining, but only falling to the level of response in non-infected mice at about week 12, or 13 when the mice were beginning to die. This increased response to TNP-schistosomules was significantly lower in nude mice, showing that the increase predominantly represents a response of T-helper cells. Mice at all but the latest stages of infection responded normally to TNP-horse red blood cells, showing that the increased T-cell response was immunologically specific (Fig. 1). Similar results have been obtained in CBA mice. In mice infected with *S. mansoni* but not injected with TNP-schistosomules, we noted that the background PFC against both TNP and SRBC began to rise from 4 weeks, possibly an indication of nonspecific B-cell activation.

In mice vaccinated with 40 formalin-fixed schistosomules a substantial but smaller helper T-cell response was seen, which was maintained for at least 13 weeks after vaccination. This suggests that both the early increase and the subsequent decline may be partly due to the living infection (Fig. 2).

In view of the fall in the helper T-cell response in the later stages of the infection (see Fig. 1), mice that had been infected 5 or 10 weeks earlier were reinfected with 40 cercariae and tested with TNP-schistosomules 7 or 10 d later. At no stage was there a significant increase in the TNP-response (Table 2). In other words, the helper T-cell response to the second infection was significantly lower than that to the first, indicating that the decline in helper T-cell activity was not due merely to the disappearance of antigens restricted to the schistosomule. We hope to establish by serum and cell-transfer experiments whether this decline in the T-cell response is caused by antibody and/or antigen, as has been shown in tumour-bearing animals<sup>11</sup> or perhaps by specific suppressor cells. One possibility is that this T-cell tolerance is associated with the deposition of eggs,

**Fig. 1** Increased carrier effect of schistosomules following infection with *S. mansoni* cercariae. The anti-TNP PFC response was measured in the spleens of mice infected with 40 *S. mansoni* cercariae and subsequently injected with 100 TNP-schistosomules or  $2 \times 10^8$  TNP-horse RBCs. The responses were assayed 4 d later, but are plotted against the day of infection. ●, (C57BL  $\times$  BALB/c)F<sub>1</sub> mice injected with 100 TNP-schistosomules; \* nude mice injected with 100 TNP-schistosomules; ○ (C57BL  $\times$  BALB/c)F<sub>1</sub> mice injected with  $2 \times 10^8$  TNP-horse RBCs. The zero time point represents the response to 100 TNP-schistosomules in non-infected mice.



**Fig. 2** Increased carrier effect of schistosomules following vaccination with dead schistosomules. The anti-TNP PFC response was measured in the spleens of (C57BL  $\times$  BALB/c)F<sub>1</sub> mice vaccinated with 40 formalin-fixed schistosomules and subsequently injected with 100 TNP-schistosomules. The broken line indicates the response of infected mice (see Fig. 1).

which release antigens known to strongly stimulate T cells<sup>3</sup>. Egg-laying does not however begin until about 5-6 weeks after cercarial penetration, whereas the reduction in helper T-cell responsiveness is already apparent by 4 weeks (Fig. 1). On the other hand, the granulomatous response to eggs in the tissues, which is also a T-cell mediated response<sup>2,3</sup>, also decreases in the course of time<sup>1,13</sup>, and it is tempting to speculate that the underlying mechanisms may be similar: in the case of our helper T cells, circulating antigens derived from the adult worm but shared with the schistosomule may be responsible for the reduced T-cell help late in the infection.

It is interesting that protective immunity in mice infected with *S. mansoni* reaches a peak relatively late (approximately 12 weeks) after infection<sup>14</sup>, at a time when the helper T-cell response has declined to the level in uninfected mice (Fig. 1). Protection seems to be at least partly dependent on antibody<sup>15</sup>, which suggests that other factors than T-cell reactivity may be responsible for its unexpectedly late development. It would clearly be of great value to be able to follow the response of the antibody-forming (B) cells during infection, with the same degree of precision as is now available for the T cells.

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# matters arising

## Similarities between "cholinergic proteolipid" and detergent-extracted cholinergic proteins

A REPORT from Changeux's group<sup>1</sup> concludes that the cholinergic receptor proteolipid<sup>2</sup> is different from the detergent-extracted cholinergic protein<sup>3</sup>. This conclusion is based on the observation that there is no immunological cross reactivity between the proteins and their respective antisera. Furthermore, confirming some earlier observations of Karlin<sup>4</sup>, they found that after affinity labelling of the receptor *in situ* with <sup>3</sup>H-MPTA, the radioactive ligand is not extracted by chloroform-methanol. They failed, however, to establish whether the cholinergic receptor proteolipid is still extractable after the affinity labelling and thus committed the same error as did Karlin previously.

In an attempt to clarify the problem we have repeated the experiments (Table 1). In membranes from *Electrophorus* we found that treatment with dithiothreitol (the preliminary step in the affinity labelling) followed by mercaptoethanol, produces a drastic reduction (61%) in the receptor proteolipid that can be extracted. By affinity labelling *Torpedo* membranes with <sup>3</sup>H-MPTA we confirmed the findings of Karlin<sup>4</sup> and Changeux<sup>1</sup> in the sense that only 6% and 14% of the radioactivity was extracted with chloroform-methanol; however in the same experiments there was a reduction of 70% and 80% in the cholinergic receptor proteolipid (Table 1). Thus an alternative and more valid conclusion to draw from the experiments of both Changeux<sup>1</sup> and Karlin<sup>4</sup> may be that the cholinergic receptor proteolipid is labelled in the membrane but is no longer extractable by the organic

solvents, although it can still be solubilised with strong detergents in aqueous solutions.

With respect to the immunological work, we can offer no explanation at present for the apparently negative results. The isolation procedure used by Changeux's group, however, is not that used by our group<sup>2</sup>. Thus the diethyl ether precipitate used by them contains only 10% of the total proteolipids and practically no cholinergic receptor proteolipid. Clearly therefore the question of the immunological comparison of the proteins must be investigated further before definite conclusions can be drawn.

It is convenient to summarise the many similarities between these two proteins. Both extraction procedures are designed to solubilise hydrophobic intrinsic membrane proteins and both proteins have been purified by affinity chromatography either in aqueous solutions<sup>4</sup> or in organic solvents<sup>5</sup>.

The number of receptor sites that can be labelled with  $\alpha$ -toxin in *Electrophorus*<sup>1</sup> and *Torpedo* corresponds closely to the amount of cholinergic receptor proteolipid that we extract<sup>2</sup>. Both isolated proteins show high affinity binding for a range of cholinergic nicotinic ligands, including acetylcholine, decamethonium, *d*-tubocurarine and  $\alpha$ -bungarotoxin. We can transfer our proteolipid to aqueous solutions using Triton X-100 and in these conditions the binding of <sup>3</sup>H- $\alpha$ -bungarotoxin can be demonstrated. Incorporation of the proteolipid into lecithin liposomes has also been achieved and the binding of  $\alpha$ -toxin in the aqueous solution has been observed. From this evidence we conclude that the cholinergic proteolipid of the electrophorus is probably identical with the binding subunit of the detergent-extracted protein.

We thank Dr J. P. Changeux for <sup>3</sup>H-MPTA and *Torpedo* tissue and Dr G. G. Lunt for helpful discussion.

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BARRANTES ET AL. REPLY—As De Robertis *et al.* point out<sup>1</sup>, the extent to which chloroform-methanol (2:1) (C:M) extracts proteolipids after treatment of the receptor-rich membranes with dithiothreitol (DDT) and reaction with <sup>3</sup>H-MPTA was not mentioned in our report in *Nature*<sup>2</sup>. It was, however, a daily experience in the laboratory that such treatments did not modify, in any respect, the dissolution of the receptor protein by detergents such as Triton X-100 or sodium cholate; but as De Robertis claims, this might no longer be true in the case of the extraction of the proteolipids by chloroform-methanol.

To our surprise, when De Robertis carries out this control he assays the proteolipid by measuring <sup>14</sup>C-acetylcholine binding after affinity labelling with <sup>3</sup>H-MPTA. From Karlin's work and from our own experience it is well established that <sup>3</sup>H-MPTA attaches covalently to the cholinergic receptor site and therefore blocks cholinergic ligand binding. On the other hand, the fact that acetylcholine binds to the proteolipid after reaction with <sup>3</sup>H-MPTA suggests that, in the presence of organic solvents, <sup>14</sup>C-acetylcholine binding is largely nonspecific (and this might equally be true for  $\alpha$ -bungarotoxin binding in such drastic conditions). Also C:M extracts about the same fraction (0.5-1%) of the total proteins from both fresh

**Table 1** Percentage reduction in the extraction of the cholinergic proteolipid from membranes of *Electrophorus* and *Torpedo* electrophorus, after affinity labelling with <sup>3</sup>H-MPTA

	$\mu$ g Receptor proteolipid per g fresh tissue		Reduction (%)
	Control	Treated	
<i>Electrophorus</i> *	5.2	2.0	61
<i>Torpedo</i> †	23.0	7.0	70
<i>Torpedo</i> †	24.9	5.0	80

The experiments were carried out as described in ref. 1. The amount of receptor proteolipid was determined after chromatography of the extract on Sephadex LH-20 in the presence of  $10^{-6}$  M <sup>14</sup>C-acetylcholine<sup>3</sup>.

\*Treated with dithiothreitol and mercaptoethanol.

†Affinity labelled with <sup>3</sup>H-MPTA (4-(*N*-maleimido)-phenyltri-<sup>3</sup>H-methylammonium).

*Torpedo* electric tissue and purified membrane fragments enriched 50–100 times in  $\alpha$ -toxin sites and containing the cholinergic receptor as 10–30% of their protein.

Nevertheless, we were able to repeat the observation made by De Robertis when the proteolipid was measured by the method of Lowry as modified by Hess and Lewin. Starting from 1 ml of a suspension of membrane fragments of specific activity 890 nmol of toxin sites per g of protein (6.6 mg protein per ml) the C:M extract contained 17  $\mu$ g protein after MPTA labelling as compared with 76  $\mu$ g protein in the absence of DTT and MPTA.

If after  $^3\text{H}$ -MPTA labelling, however, the membrane fragments are exposed to an oxidising reagent such as 1 mM 5,5'-diithiobis-(2-nitrobenzoic acid) (DTNB) C:M extracts 46  $\mu$ g of protein from 1 ml of membrane suspension, a value closer to that found before DTT treatment. In both conditions, that is after  $^3\text{H}$ -MPTA labelling and with or without DTNB treatment less than 2% of  $^3\text{H}$ -MPTA (and not 6% and 14% as mentioned by De Robertis) is extracted into the C:M. Therefore, -SH reagents markedly modify the extractability of the proteolipids by C:M but show no effect on that of the radioactively labelled receptor.

During these control experiments, we noticed that DTT added to the C:M extract of proteolipid considerably enhances its transfer into an aqueous 1% Triton X-100 phase (the ratio of aqueous to organic phase of 2.6:13.0 becomes 16.0:1.6  $\mu$ g protein per ml in the presence of 1 mM DTT). Nevertheless, the DTT-solubilised proteolipids extracted by Triton X-100 still do not bind  $^3\text{H}$ -toxin from *N. nigricollis* in the presence of physiological Ringer solution in the conditions routinely used for receptor assay.

Finally, a comparison has been made between native receptor-rich membrane fragments and membranes which have been extracted with C:M. SDS-polyacrylamide electrophoretograms of both preparations reveal the presence of the 39,000–41,000 daltons band characteristic of the cholinergic receptor protein. Electrophoretograms of membranes labelled with  $^3\text{H}$ -MPTA show this to be the only band to be radioactively labelled and confirm its identity as the receptor.

These observations give additional support to one of our initial conclusions; that the C:M-extracted cholinergic proteolipid shows little if any relationship to the now well identified cholinergic receptor protein in its detergent-extracted form.

## Climate in the 1970s

A FEW words near the end of our letter<sup>1</sup> headed "Climatic reversal in northern North Atlantic", seem to have resulted in our diagnosis of recent climatic shifts being so widely misrepresented elsewhere that some further statement is necessary.

Our letter gave a diagnosis of what had been happening in the North Atlantic-European sector between 1971 and 1975, and was explicitly not a forecast. That diagnosis was, and is, valid, though it was probably not advisable to use the words "little ice age" to describe two decades of colder climate, and it was certainly not advisable to suggest that that episode was over just because of a run of four or five mild winters in Europe, when the other seasons (in spite of August 1975!) continue mainly cold, and the generally lower level of temperatures over the Arctic above 70°N, which set in about 1961, persists.

What has happened since 1971 may be largely attributed to a change of the prevailing wave positions in the circumpolar vortex, even though we do not yet know what causes such shifts involving the centre of the polar cap itself. In 1971 the Canadian Arctic became, for the first time, involved in the cooling that set in sharply in other sectors of the far north in 1961, and, just as sharply, the coldest centre was transferred to the Canadian sector. This meant that, there was a great increase in the south to north thermal gradient between the western Atlantic and northern Canada, particularly in the winter months, resulting in a great increase in the energy of the atmospheric circulation over the North Atlantic, driving mild air towards Europe and more saline water to Iceland—as M. Rodewald (unpublished) has pointed out. But these conditions do not seem to have penetrated as far into the Arctic as during the general Arctic warming in the 1920s and 1930s, and it is not yet clear whether the mechanism described since 1971 is capable of restoring conditions there to what they were before 1960. Doubts on this are strengthened by observation that the centre of the polar cold regime and the waves in the circumpolar vortex returned to their 1960s' positions for about six months around late 1973 and again in 1975. The ice then again increased in the sector east of Greenland, and once more approached the coasts of Iceland in July and August 1975, perhaps for the first time this century in those months.

S.-A. Malmberg informs me that in June 1975 off north Iceland the salinity anomaly was slight, only  $-0.03\text{‰}$ , though the temperature anomaly was  $-1.60^\circ\text{C}$ . No influx of the Atlantic watermass into northeast Icelandic waters was observed in June 1975. The ice conditions were "relatively unfavourable", and by July 1975 there was ice in the East Iceland Current. In June 1975 both the polar and

Atlantic currents were weak in the north Iceland region. Sometimes both these currents are strong (as in 1964 and 1968), and sometimes one is strong and the other weak: no relationship has been found between them.

Recent investigations<sup>2–4</sup> imply that the great variations to which the volume of polar water transported by the East Greenland Current and the southward penetration of this water in the East Iceland Current are prone, may be one of the most interesting and important aspects of climatic change, affecting alike the development of major ice ages, the little ice age of recent centuries, and the cooling since the 1950s.

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## Lattice absorption in small particles

LUKES<sup>1</sup> has suggested that the width of the lattice absorption peak for small particles of ionic solids at very low temperatures would be dominated by collisions of phonons with the surfaces. The estimate given for the phonon collision time,  $\tau \sim R/v$ , where  $R$  is the particle radius and  $v$  is the velocity of sound in the bulk, is, however, applicable to acoustic phonons only. This collision time is not relevant to the phenomenon of lattice absorption, which is due to the long-wave optical phonons. The collision time  $\tau$  enters the calculations of lattice thermal conductivities at very low temperatures, when all other possible scattering mechanisms become ineffective. This is a well known boundary effect which has been investigated extensively<sup>2,3</sup>.

As regards lattice absorption by small particles, many experiments have shown the absorption peak to be considerably broader than that calculated by using the bulk value of the damping constant  $\gamma$  appearing in the expression for the dielectric constant<sup>4,5</sup>. For small NaCl and MgO crystallites ( $R \lesssim 1\mu\text{m}$ ) it has been estimated<sup>6</sup> that the damping parameter increases beyond its bulk value by a factor of  $\sim 2.5$ . No fundamental calculations of  $\gamma$  in small particles, analogous to those which exist for bulk crystals<sup>7</sup>, have so far been performed.

R. RUPPIN

Soreq Nuclear Research Centre,  
Yavne, Israel

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# Landmarks in North Sea geology

There is a comparable breadth of technical information. This ranges from the trans-European stratigraphic syntheses presented in papers by the Ziegler brothers, through the many field case histories (documented by



# Europe in the Sixteenth century by A. Ortelius

Courtesy The Mansell Collection London

There is no doubt that the discovery of North Sea oil has been the biggest stimulus to British geologists since the advent of William Smith. This volume provides a landmark in the history of North Sea geological studies and may be the definitive text and sourcebook for more than a decade. It provides an excellent insight into the thought processes and extent of technical knowledge required by a practising petroleum geologist. This book may demonstrate to many geologists outside oil companies that the search for petro-

At £16 for 501 pages, this book is worth every penny. **R. C. Selley**

The papers explaining how oil wells are drilled and fields developed and produced will, I fear, make heavy reading for the non-specialist to whom they should have been addressed. An overall impression of complex problems solved with sophisticated equipment and with considerable attention to preventing oil spillage is conveyed. Reading these papers, however, the non-specialist would have to be very persevering to understand what a blow-out is, how it occurs, who can prevent it and with what equipment.



The papers on oil pollution will be of much greater value to the non-specialist. They are no less technical than the oil industry papers but are neatly divided into the effects on Fisheries, Shore Life, Birds and Long Term Low Level Exposure in all areas.

The problems associated with analysing the effect of one pollutant, oil, in the marine environment are expressed in all papers, and admirably summarised in one, as follows: "Chronic pollution of marine waters by oil from numerous sources is widespread but hard to quantify. It is also difficult to specify its biological effects, since these can often be deduced only from laboratory experiments in conditions which are far from natural. In the field, it is rarely possible to exclude the effects of other pollutants or adverse natural conditions."

Two papers provide valuable data on the role of Government and the oil industry in oil spill clean up.

This volume will be a valuable reference to those interested in the problem of oil and its effects on the marine environment. **P. Selwood**

*Geology of the North-West European Continental Shelf.* Volume 1. By D. Naylor and S. N. Mounteney. Pp. 162. (Graham Trotman Dudley; London, 1975.) £6.50. Volume 2. By R. M. Pegrum, G. Rees and D. Naylor. Pp. 225. (Graham Trotman Dudley; London, 1975.) £8.50.

WITH the increasing level of effort now being put into the exploration and exploitation of the Continental Shelf surrounding the British Isles, a large number of non-petroleum specialists have become involved in the work. Bankers, lawyers, accountants, economists, and professionals in many disciplines are finding that an elementary understanding of the geology of the various areas in which their companies are working is a distinct advantage. And it is for such professionals that these books were essentially compiled.

It is not quite clear why the book is in two volumes particularly as both contain together only 380 pages. Nevertheless the division between the books is clear enough—the first dealing with relatively unexplored western basins of the Shelf, the second being confined to the North Sea area. If a division has to be made then this at least is a logical approach.

Assuming their intended market, both volumes tend to assume very little geological knowledge, but there are times when without it the layman will be stretching a little. Volume 1 begins by introducing the concept of plate tectonics in an attempt to place the

## Biochemistry of hormone action

*Biochemical Actions of Hormones.* Volume 3. Edited by Gerald Litwak. Pp. xvi+415. (Academic; New York and London, August 1975.) \$36; £18.

READING this book straight through it becomes increasingly obvious that the real breakthroughs in the understanding of hormone action will only come when the tools provided by molecular biology and genetics are brought to bear on the problems. Unfortunately, not all the hormone-responsive systems currently under study are amenable to

British Isles in a regional framework. To a geologist the way in which this is done may seem a little glib but the message may get across nonetheless. By reference to geophysical and bottom sampling data acquired in each of the western basins together with a resumé of the inferred stratigraphy, a generalised picture is built up of the basins stretching from the Channel to the west of Shetland. The amount of information in each chapter varies with the amount of information published, but nevertheless for the audience intended there is sufficient data to give a reasonable idea of the variations in structure and rock type between each of the basins considered.

A more systematic treatment, however, with maps, cross sections and stratigraphic tables, would have enabled comparisons to be made rapidly, as would mention of the relationships between onshore and offshore geology, even in diagrammatic form. The account of the palaeogeography and the evolution of western Britain, brief though it is, seems somewhat misplaced. Indeed it might be said that the book tends on occasion to forget its audience. What will be useful to the layman, however, are the brief notes on how oil is trapped, and explanation of how a seismic survey is conducted and how a well is drilled. These are partially repeated in the second volume together with a glossary, but they perhaps bear re-enforcement.

The second volume is concerned mainly with the North Sea and the information it contains is based on factual material, much of it released for the first time at the Conference in London last November, and which is drawn on freely. The stratigraphy is described systematically from the basement to the Tertiary system, and sadly the procedure of describing Basins is not adopted in this volume as it was in

these approaches and very often when these tools would prove useful they are not applied. The articles in this book serve to illustrate this point.

Molecular and genetic studies are obviously much easier where the gene product affected by the hormone can be identified; the chapter by Schimke *et al.* provides an elegant example of molecular biology being used to study ovalbumin induction in the chick oviduct. This chapter appears next to another (on the same topic) by Rosen and O'Malley which has an annoying amount of overlap. Indeed this is a general fault with the book: half of it is concerned with steroid hormones, and the central dogma of steroid action is repeated several times. But some of

the first. In many ways this method is a much more convenient one of describing an area which geologically has a very complex history.

The "field data sheets", which is effectively what they are, will be appreciated, however, by the layman as they condense a considerable amount of information very concisely. Each field is presented in a similar manner each having a map and cross section together with pertinent information on the field. The stratigraphic information which has been derived from studies of the fields is incorporated into the accounts of various geological systems and thus the fields are placed in their geological perspective. Because the book does not use the Basin approach of the first volume, however, the significance of the various discoveries (for example, the predominance of gas fields in the southern North Sea Basin) might go unnoticed.

It may be that it is too early in the history of the exploration of the Continental Shelf to attempt the synthesis which is presented in the second volume. There is still a great deal of information that remains confidential, and authors are unable to construct the geological cross sections and maps which would make all the difference to unravelling the history of these areas and which are so essential to an elementary understanding of the geology.

In many respects the two volumes are complementary, one deals with essentially untried areas the other with proven and established Basins. They both contain a considerable amount of compiled information—albeit very generalised. It is a matter of choice and of the reader's expertise as to whether one goes to the original text (the proceedings) or to this concordance. **J. R. V. Brooks**

these articles are good. There is an excellent review of the action of oestrogens by Katzenellenbogen and Gorski, although it is somewhat marred by the primitive techniques used to analyse oestrogen-induced RNA synthesis.

There are three chapters dealing mainly with hormone receptors; that by Hollenberg and Cuatrecasas is a particularly useful introduction to peptide and catecholamine receptorology. One other chapter deals exhaustively with the glucocorticoid receptor and another discusses the evidence for a specific nuclear receptor for thyroid hormones.

Any genetic approach to the problem of hormone action presupposes that hormone-mediated events can be studied in cultured cells. The problems of establishing hormone responsive cells in culture is discussed in two chapters,

one by Armelin (which mentions some of the pitfalls) and a disappointingly brief chapter by Sato. In it he discusses the role of serum in cell cultures and comes to the conclusion that a whole new series of peptide hormones are about to be discovered.

The hardest task in reviewing this book is trying to decide who it was aimed at. Although some of the topics treated have not been reviewed previously, libraries are becoming full of books which serve as a vehicle for yet more reviews on the same topics. It might be more useful to have more shorter reviews with good references so that the reader has enough information to go to the relevant literature if he wishes. This book is, however, good of its genre and provides a relatively painless entry into some of the biochemistry of hormone action.

Robert Shields

differ according to the broadscale environment and to the geographical zone, season of the year, synoptic weather situation and so on. The subject is at the same time eminently practical and useful but unfortunately general principles do not get us very far and empirical studies for local application need to be made in every part of the world; the literature becomes ever more voluminous as many hundreds of worthwhile studies are published every year. How does one set about reducing such material to a textbook for a university course. Professor Yoshino is one of the few who have tried.

A short opening chapter on terminology is followed by 25 pages of 'History of Research', just enough to remind students that the subject is not a new one. In chapter 3 of 142 pages, climate is related to the surface, flat land, city, forest, seashore, lakeshore, riverbank, including some fundamental boundary layer theory which seems either too much or too little. A chapter of similar length concerns 'topography', here implying mountains, hills and valleys, and its relationship to climatic elements; the next is entitled 'Local Airstreams and Weather' and relates the weather (rather than the climate) with synoptic pattern, local fronts, and local winds, fohn, bora, and the like. The sixth and last shorter chapter is headed 'Local and Microclimate and Nature' which could mean anything but turns out to be just a taste of applied climatology, with geomorphology and plant ecology, and provides a place for a pet subject of the author, wind shaped trees.

A broad qualitative knowledge of meteorology and its basic principles is assumed and just occasionally mathematics finds a place. The Japanese book production is excellent and would do credit to the best English academic publishers although with sufficient minor misprints and infelicities in English to call for a little indulgence. It is a book to be acquired by every specialist library although the inevitable bias towards Japanese climate and Japanese literature makes it unsuitable as a coursebook outside Japan.

I have never taken readily to the textbook, however, which in its anxiety to acknowledge its sources reads at times more like an annotated bibliography. Nearly 1,000 different authors earn some 1,200 references and yet the selection from the much vaster world literature still seems almost arbitrary. A text for students which digests the material and is sparing with the 'credits' can be briefer and cheaper, more cogent, more instructive and certainly not so soon dated, although the scholarship may be less obvious.

R. C. Sutcliffe

## Analysing sediments

*Archaeological Sediments: a Survey of Analytical Methods.* By Myra L. Shackley. Pp. 159. (Butterworths: London and Boston, Massachusetts, June 1975.) £7.00.

THIS book is a compendium of methods for the description and analysis of inorganic sediments laid down by wind and water, and for the handling of field and laboratory data. A large part of the book deals with particle size analysis and is a thorough and reliable guide to techniques in this field, but there is very little discussion of experimental errors, and a spurious precision of measurement is recommended, given the sampling strategy proposed. Interpretation of field observations and experimental results receives very little attention, and examples of the use of the methods are presented with uncritical acceptance of their suitability to the problems in hand and of the value of the results. Outside the field of traditional sedimentology, selection of sources and coverage of techniques is erratic, and misleading advice and information appears. In the confused discussion of the relationship between soils and sediments no mention is made of mineral alteration and secondary mineral formation; organic components of sediments, although their study is a separate field, surely require discussion as participants in the processes of sedimentation and postdepositional alteration. Most archaeological sediments are complex mixtures of mineral and organic materials of

diverse origin and history, laid down in intricate interstratification by people and animals as well as by the inanimate agencies, and their study demands a synthesis of methodology derived from many branches of field and laboratory investigation. This book understates the limitations of the contribution its methods can make in deriving information about human environment and behaviour from the deposits left by or related to human activity. It provides a useful short cut to the standard literature of sedimentology, but fails to provide the archaeologist or the scientist working with him with the help in formulation of questions, selection of appropriate techniques and interpretation of results which he badly needs.

Susan Limbrey

## Small-scale meteorology

*Climate in a Small Area: An Introduction to Local Meteorology.* By Masatoshi M. Yoshino. Pp. xvi+549. (University of Tokyo: Tokyo; International Book Distributors: Hemel Hempstead, Herts, April 1975.) £16.

PROFESSOR YOSHINO has provided a successful textbook on small-scale climatology, an area which does not lend itself readily to systematic presentation. It is a multidimensional subject in which the range of physical variables—pressure, temperature, humidity, cloudiness, sunshine, rainfall, wind and others—are related to a comparable variety of physical features—hills and valleys, land and sea, rivers and lakes, trees and forests, towns and cities—all of which have effects which

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**Edward Arnold**

25 Hill Street, London  
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## Developmental controversy

*Cell Cycle and Cell Differentiation.*

(Results and Problems in Cell Differentiation: a Series of Topical Volumes in Developmental Biology, Volume 7.)

Edited by J. Reinert and H. Holtzer.

Pp. xiii+331. (Springer: Berlin and New York, 1975.) DM69; \$29.50.

As editors of *Cell Cycle and Cell Differentiation* Reinert and Holtzer have put together chapters on some of the currently most interesting topics in developmental biology. This monograph can be roughly divided into two parts: the first four chapters deal with different cell types whose differentiation depends on a critical stage in the cell cycle, the remaining chapters either equivocally touch on the dependence of differentiation on the cell cycle itself, or argue that it may be irrelevant to differentiation.

The book begins with a chapter on myogenesis by S. R. Dienstman and H. Holtzer, the latter being currently one of the most prominent protagonists of the idea of the 'quantal cell cycle' as a key to understanding cellular differentiation. The next three chapters also echo the same theme of coupling of cell division or cell cycle to the differentiation of erythroid cells (H. Weintraub), of neuronal specificity (R. K. Hunt) and neurogenesis (C. H. Phelps and E. Pfeiffer). Those authors by and large agree with Holtzer's concepts but also urge caution before all the conclusions regarding lineage-dependence of differentiation are definitively accepted. The question of the mechanism of quantal cell cycle is however left unanswered, nor is it clear if such a cycle plays a 'causal' or a 'permissive' role in the expression of a developmental programme.

R. C. King gives a good account of the exploitation of genetics and hormones (particularly ecdysone) in evaluating the role of cell division during oogenesis in *Drosophila*, but avoids a clearcut conclusion about the causal role for the cell cycle or cell division. The book then takes a major turn with two short chapters by P. Lawrence and J. B. Gurdon who come out against quantal mitosis as essential for differentiation. Lawrence uses his work on pattern formation in larval and adult cuticle development in the insect, *Oncopeltus*, a process regulated by juvenile hormone, to argue that whereas asymmetric cell divisions may generate diversity it is not certain that symmetric mitosis leads to differentiation of daughter cells. Gurdon turns to his group's work on nuclear transplantation to argue that cell division

is not essential for all differentiative processes. He proposes that what may be important is cyclic condensation and swelling of chromosomes; the latter would pick-up a new set of cytoplasmic regulatory proteins during their postmitotic decondensation. It seems to me that there is room for Holtzer's quantal cell cycle idea as well as those of Lawrence's and Gurdon's and that these need not be mutually exclusive.

Four chapters depict the importance of studying differentiation in simpler organisms before attempting to interpret the more complex phenomena of animal cells. These include morphogenesis in the prokaryote, *Caulobacter* (N. B. Wood and L. Shapiro), plants in general (F. Meins, Jr), plant tumour formation as a differentiative process (A. C. Braun) and cell differentiation in *Neurospora* (R. E. Nelson, C. P. Selitrennikoff and R. W. Siegel). None of the authors of these chapters provide strong evidence for or against the idea of a quantal cell cycle as the basis of cell differentiation and hint that many morphogenetic changes can be dissociated from growth and cell division. Meins, after discussing mathematical analysis of several well known plant differentiative kinetic models, emphasises the well-known totipotency of differentiated plant cells which strongly suggests that cytodifferentiation results from epigenetic rather than permanent genetic modifications. Tsanev takes the reader back to animal cells with a detailed and fresh analysis of the very complex changes associated with hepatic regeneration, a very popular model for temporal analysis of functional changes and DNA synthesis but one which does not clarify the role of the cell cycle in differentiation and goes on to suggest that one should separately consider the activities of 'tissue-specific' and 'mitotic' genes. Th. W. Borun makes a plea for considering histones as important for early cell differentiation and not to regard them merely as 'glue' for keeping inactive genes repressed. He distinguishes the simple multiplicity of histones from the microheterogeneity generated by their phosphorylation, acetylation and methylation particularly of histones.

For students of developmental biology, *Cell Cycle and Cell Differentiation* will be most valuable for bringing together views of some of the most original workers in this field, but the controversy it generates about the essential role of quantal cell division may be more apparent than real. The monograph once again shows how far we still are from, or even how futile it may be to search for, a unified hypothesis for the oldest unsolved problem of biology.

**J. R. Tata**

# obituary

**Kasimir Fajans** was born in Warsaw, Poland, May 27, 1887, and died in Ann Arbor, May 18, 1975. He went to school in Warsaw, to university in Leipzig, and then to Heidelberg where he did his Ph.D. work, and also won the Victor Meyer Prize (1909). He spent the next two years as a postdoctoral student at the Technische Hochschule in Zurich, where he became acquainted with Albert Einstein, and at the University of Manchester, where he worked with Lord Ernest Rutherford. After six years at the Technische Hochschule at Karlsruhe, he moved to Munich, and became the director of the Institute for Physical Chemistry in 1932. He first visited the United States in 1930 as the Baker Lecturer at Cornell University, and returned in 1936 as Professor of General and Physical Chemistry at the University of Michigan, where he remained for the rest of his career.

His major work was on radioisotopes, where he discovered the branching of radium transformation series in 1911, established the radioactive displacement laws in 1913, found the first isotope of the 91st element in the Periodic Table and discovered the precipitation rule of radio elements. Less well known, but equally fruitful, was his prolific research on other topics in chemistry. A partial list would have to include: the heat of hydration of gaseous ions, thermo-chemistry, chemical binding, volumetric analysis by adsorption indicators, theory of glasses, and the partial separation of D- and L-stereo-chemical isomers by asymmetrical catalysts. He was the author of five scientific books; and remained active long after his formal retirement, the last of many awards given to him, the Gold Award for 1975 of the Affiliate Council of the Engineering Society of Detroit, was bestowed only a few months before his death.

Professor Fajans married Salomea Kaplan, in 1910. She survives him together with their two sons Edgar W. and Stefan S., four grandchildren and two great grandchildren.

Thomas M. Dunn

Like many men of modest physical stature, **Hamilton Hartridge** who died on January 13, 1976, at the age of 89, was a dynamo. He used to produce

ideas at the rate of a normal person's heart-beat, a faculty which dove-tailed with his nineteenth century many-sidedness, but ultimately led to his professional evaporation.

After completing his medical studies he stayed at King's College Cambridge till 1926. It was there that he did his best scientific work. He collaborated with Roughton in the classical analysis of the composition of blood in relation to various levels of oxygenation the study was furthered by Hartridge's ingenious invention of the reversion spectroscope, suitable for an estimation of the movement of haemoglobin bands. He suspended silk threads from the ceiling of his room at distances just greater than a bat's wing-span, and showed that, though the bats flew around, not a single thread was torn. He provided an optical system enabling Rutherford and Geiger to get on with their experiments counting  $\alpha$ -particles. Later he buttressed Helmholtz' theory of hearing. He applied physical principles to the theory of visual resolution in the early twenties, was elected to the Royal Society and appointed subsequently—if not consequently—to the chair in physiology at Barts in 1927. This he occupied till 1947.

Paradoxically, his name is best known in the world of vision undoubtedly because of his literary rather than his scientific output. His crucial experiment on accommodation in the cat is forgotten. But his quixotic attacks on the validity of Young's trichromatic theory of colour vision raised enough dust for even some present-day authors deeming it necessary to flick a duster. Hartridge's lively brushes at the Physiological Society and the Colour Group of the Physical Society (as it then was) intrigued even laypersons.

Explicitly presented in a volume of Transactions of the Royal Society, affectionately known as the Yellow Peril, his polychromatic theory is alien to British sensory physiology in being unquantified and therefore untestable. The Medical Research Council set up a Vision Research Unit in 1947 under Hartridge's direction so that he might have a chance to prove his ideas. He failed. Yet he managed to attract a handful of people who have directly and indirectly stimulated vision research in the last thirty years both in this country and abroad. This should not be forgotten even though it is unlikely to be engraved on any stone.

R. A. Weale

**Dr R. W. B. Nurse**, a former Head of the Materials Division at the Building Research Station and a world authority on cement chemistry, died on November 13, 1975, aged 62. He received his degree in Physics from London University in 1934 and began at the BRS, which he joined in 1932, his fundamental work on inorganic building materials including the thermodynamics of multicomponent systems. Early in his career he invented with F. M. Lea (later Sir Frederick Lea) the Lea-Nurse apparatus for determining the specific surface of powders by an air-permeability method. He made major contributions to Portland and slag cement chemistry, for which he was awarded the D.Sc., London, in 1954, and contributed principal papers to the 4th, 5th and 6th International Symposia on the Chemistry of Cement. He was probably best known for his work on the manufacture of cement from impure limestone which led to the setting up of a thriving cement industry in Uganda, the first anywhere in the world to use phosphatic limestone. His work was recognised in 1963 by the award of the Sir George Beilby Medal. He also initiated at BRS studies of fibrous composites and crystallised glasses. In 1965, in recognition of his outstanding research abilities he was awarded special merit promotion to Deputy Chief Scientific Officer, which enabled him to reduce his administrative responsibilities and to concentrate on research. Between 1965 and his retirement in 1973, he continued his work on fibrous composite materials, contributing to the understanding of the properties and fracture mechanics of glass fibre cement composites in particular. He had been President of the Mineralogical Society and was an honorary member of the Permanent Committee of RILEM (Réunion Internationale des Laboratoires d'Essais et de Recherches sur les Matériaux).

Ron Nurse was a man of very wide-ranging interests and talents: he took a particular interest in the training of young people, for instance as a member of the Board of Governors in local schools and colleges. Among his many qualities his generosity of mind, modesty and his willingness to help others were outstanding. He leaves a wife, a son and a daughter by whom, as well as by his colleagues and friends, he will be greatly missed.

Witold Gutt



# announcements

## Appointments

**Sir Denys Wilkinson**, Professor of Experimental Physics at the University of Oxford, has been appointed Vice-Chancellor of Sussex University.

**Professor John B. Goodenough**, Leader of the electronic materials group at M.I.T., has been appointed Professor of Inorganic Chemistry at the University of Oxford.

## Awards

The Geological Society of London has announced the following awards for 1976:

The **Wollaston Medal** to **Sir Kingsley Dunham**, Foreign Secretary of the Royal Society, an authority on mineral occurrences in Northern England.

The **Murchison Medal** to **Professor R. A. Howie**, King's College, London, for his research on pyroxenes and other minerals and his contribution to teaching.

The **Lyell Medal** to **Mr W. B. Harland**, University of Cambridge, for work in Spitsbergen, contributions to stratigraphy and the geology of tillites and work on orogenesis.

The **Prestwich Medal** to **Professor W. W. Bishop**, Queen Mary College, London, for his studies of mammalian palaeontology and the stratigraphy and geomorphology of Miocene-Pleistocene deposits in East Africa.

## International meetings

March 1, Deadline for **Experimental Use of Algal Cultures in Limnology** to take place on October 26-28, in Sandefjord, Norway (Norwegian Institute for Water Research, P.O. Box 333, Blindern, N-0slo 3, Norway)

March 25-26, A symposium on **The Zoological Society of London 1826-1976 and Beyond**, to be held at the Meeting room of the Zoological Society (Assistant director of Science (UM), Zoological Society of London, Regent's Park, London NW1 4RY, UK)

March 30-April 2, **Sixth Conference on Molecular Spectroscopy**, University of Durham (Institute of Petroleum, 61 New Cavendish Street, London W1M BR, UK).

March 30-April 2, **The Synapse**, St Andrews, Scotland (Dr G Cottrell, The Wellcome Laboratories, The Gatty Marine Laboratory, St Andrews, Fife, UK).

## Person to Person

**Joseph Lister**, a new biography. I wish to consult letters, diaries, related materials held by friends, former patients, family, especially descendants of Lister's sisters, Isabella Sophie Pim and Jane Harrison, and of James Syme, the Scottish surgeon, Richard B. Fisher, 26 St Paul's Road, London, N1. 01-226 2765.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

March 31, Deadline for attendance at an **International Symposium on Migraine**, London (The Director, The Migraine Trust, 23 Queen Square, London WC1N 3AY, UK).

March 31-April 2, **Intraocular Foreign Body and Metallosis**, organised by the German Ophthalmological Society, Cologne (Professor Dr H. Neubauer, Universitäts-Augenklinik D 5 Cologne 41, Joseph-Stelzmann-Strasse 9).

April 1-2, **A Symposium on Diabetes and Other Endocrine Disorders During Pregnancy and in the Newborn Infant**, New York City (Professor Maria I. New, Cornell University Medical Center, 525 East 68th Street, New York, N.Y. 10021).

April 5-8, **mRNA: The Relation of Structure to Function**, Gatlinburg, Tennessee (Elliot Volkin, Chairman, Organising Committee, 1976 Biology Research Conference, P.O. Box Y, Oak Ridge, Tennessee 37830).

April 5-6, **Microscopy of Organic Sediments**, Oxford (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford OX4 1AJ, UK).

April 5-8, **Anglo-Dutch Wood Anatomy Meeting**, Oxford University (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford OX4 1AJ, UK).

April 5-9, **Fifth European Ophthalmology Congress**, Hamburg (The Congress Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, The Netherlands).

April 5-9, **EMBO Workshop on Thermodynamics and Calorimetric Studies on Biological Systems**, Santa Mar-

gherita Ligure, Genoa (Dr Giovanni Rialdi, c/o Centro Chimica Fisica di Macromolecole Sintetiche e Naturali, Consiglio Nazionale delle Ricerche, Istituto di Chimica Industriale, Via Pastore, 3-16132 Genova, Italy).

April 5-9, **The Annual Chemical Congress of the Chemical Society and the Royal Institute of Chemistry**, Glasgow (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

April 6-8, **Components of Protein Synthesis**, Cambridge (Dr A. R. Fersht, M.R.C. Laboratory of Molecular Biology, University Postgraduate Medical School, Hills Road, Cambridge CB2 2QR, UK).

April 6-8, **Advances in Mechanisms of Drug Metabolism and Species Differences in Drug Metabolism—from Microbe to Man**, Guildford (Dr L. J. King, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, UK).

April 6-9, **Biology and Systematics of Colonial Organisms**, Durham (Dr B. Rosen, Department of Palaeontology, British Museum (Nat. Hist.), London SW7 SBD, UK).

April 7-9, **A Symposium on Endocytosis and Intracellular Digestion**, organised by the British Society for Cell Biology/Lysosome Club, Nottingham (Dr T. R. Ricketts, Department of Botany, School of Biological Sciences, University of Nottingham, Nottingham NG7 2RD, UK).

April 8-9, The British Institute of Radiology announces its **34th Annual Congress and Scientific Exhibition**, London (The General Secretary, The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG, UK).

April 8-9, **Moisture in Solid Materials in the Process Industries**, jointly organised by SIRA and The Koninklijk Instituut van Ingenieurs (Netherlands), Delft (Mrs R. G. Keiller, SIRA Institute Ltd, South Hill, Chislehurst, Kent BR7 5EH, UK).

April 8-9, **The Ordinary Meeting of the Royal Astronomical Society**, Manchester (The General Secretary of the Royal Astronomical Society, Burlington House, London W1V 0NL, UK).

April 30, Deadline for abstracts, **The Stratosphere and Related Problems**, Logan, Utah, on September 15-17 (Dr Wesley T. Huntress, Jr, Executive Secretary, Jet Propulsion Laboratory, M/S 183-401, 4800 Oak Grove Drive, Pasadena, California 91103).

nature

February 26, 1976

## Your man in Whitehall

TWICE in the past three months the absence of a Chief Scientific Adviser to the government has been pointedly remarked on. Sir Alan Hodgkin, retiring President of the Royal Society, drew attention to the vacant post in his farewell address in November. Now the Commons Select Committee on Science and Technology, in an exchange of letters with the Prime Minister, brings the issue to the surface again. But are those who ask for one person to head government's central machinery for co-ordination of policymaking really clear that the system would thereby work any better than it does at present?

The post of Chief Scientific Adviser based in the Cabinet Office has been vacant for almost two years. Sir Alan Cottrell, having served for his five-year term, returned to academic life. His deputy, Dr Robert Press, a career civil servant with a background in nuclear matters, took over responsibility for science and technology without being named as chief adviser. Dr Press retires this summer; hence the rumblings.

The Select Committee says that in the past its suggestion of a ministry for research and development was brushed aside because the Chief Scientific Adviser was already said to be responsible for ensuring adequate co-operation and co-ordination between departments. In the absence of such an adviser, the committee says, "[we] wonder whether assurances given by successive governments about the co-ordination of research and development continue to represent government policy . . . failure to fill the vacancy has caused considerable dismay in the scientific community, and has raised doubts about the sincerity of the government's commitment to the importance of an independent scientific voice in the formulation of policies at the highest level."

In reply, the Prime Minister emphasises that Dr Press's appointment was intended as transitional, but that he has in mind stronger central co-ordination—though the appointment of Chief Scientists in many ministries "emphasised the increasing importance attached to scientific advice and underlined the impracticability of providing this from one central point. The creation of the Central Policy Review Staff as a multidisciplinary body of advice to the Cabinet also added a new dimension." Mr Wilson missed an excellent opportunity very recently of further sounding out views on science in government by devoting most of a speech to the Parliamentary and Scientific Committee to fulsome praise for the National Research Development Corporation (repeated almost verbatim from the Blackett Memorial Lecture of three months earlier), and by relegating questions of science in government to a few sentences on the

difficulty of pleasing everyone all the time. The question of advisers in general was passed off with a joke about an advisory bull belonging to FAO.

The real trouble with the post of Chief Scientific Adviser is that he is placed in such an ambiguous position. The Select Committee's letter refers to at least four functions:

- responsibility for interdepartmental coordination and co-operation
- co-ordination of research and development
- an independent scientific voice in policy formulation
- articulation of scientific advice to the Cabinet.

The academic community would undoubtedly add a fifth—provision of a direct channel for academic scientists to have access to government particularly when they held views which diverged from those expressed by civil service scientists. Industrial scientists might add further functions. The job begins to look attractive only to a megalomaniac or a superman.

Further, neither of the last two advisers exactly raved about the job after their departure; they certainly gave the impression that they found it rather frustrating. If famous names were now to be canvassed for the post, the government would have a lot of arm-twisting to do. And if the scientific community has been expressing dismay at the lack of a Chief Scientific Adviser, it has been doing so in a most *sotto voce* way.

Mr Wilson declares that he wants stronger central co-ordination but can do without the centralised advice, so he will probably look for a sound scientific administrator from within the civil service rather than a famous name from without. If he views the civil service as the sole source of scientific input to the government, this makes sense. But hundreds of millions of pounds are spent each year on nurturing science and technology both in academe and industry. Much of this expenditure will not result in anything that the government desperately needs to know about, but the existence within the Cabinet Office of some sort of external liaison office accessible to research councils, industrial organisations and individual scientists might well ensure that when matters of importance do turn up, they do not get tangled up in Whitehall bureaucracy and interdepartmental rivalry.

The post of Chief Scientific Adviser should no longer be retained. But when Mr Wilson recruits his top co-ordinator for departmental science he should also hire a good scientist from outside the civil service as liaison officer with the outside world.



## Change and consequence

In a second concluding article, **Peter Pockley** looks at Australian science and the recent election.

SCIENCE was not a starter in the public campaigning for the December election although there was plenty of activity behind the scenes. The Liberal Party did, however, make three mentions of science which were important in the light of subsequent events. This made the score Liberals 3, Labour 0, for the Labour campaign concentrated on dramatising the constitutional issue of the powers of the Senate and the essentially political actions of the Governor General in bringing down the popularly elected government.

The first mention came in Mr Fraser's policy speech, in which, as an item of evidence for ineptness in the Labour administration, he took a passing swipe at the research grants controversy. The second item was the Liberal Party's issuing of a statement on science policy in which the Department of Science retained a central role. Mr Fraser's acceptance of any science policy is a political turn-around. In 1968, as Minister for Education and Science, he had argued in a major speech before an Academy of Science meeting that attempts to define a science policy were futile. This *volte face* paled into insignificance, though, before some others which Mr Fraser underwent but which he successfully played down during the campaign: he had, for instance, been one of the strongest advocates and administrators of compulsory military service and of the ultimately futile expedition by Australian forces, including conscripts, to the Vietnam war, policies which he could not afford to embrace in 1975.

Mr Fraser got away with such inconsistencies by developing a form of non-speak which he used successfully to deflect all specific questions with varying combinations of a few generalised, qualified or conditional phrases. This technique, which tied him down to almost no action of detail or permanent substance, was aggravating in the extreme to working journalists and political opponents alike, but such was the strength of feeling he generated against Labour that he had no cause for deigning to answer questions with facts or commitments. An example of this approach was provided by the third mention of science in the campaign.

Only five days before the election, Mr Fraser personally issued a statement designed to quell speculation that the Australian Science and Technology Council (ASTEC) would be abandoned or greatly modified by a Liberal government which had arisen from some

remarks on a radio programme by the then Liberal spokesman on science, Mr Eric Robinson. The statement largely affirmed the objectives defined for ASTEC by Labour (although not by name, of course) and went on to say that it will report directly to the Prime Minister. This plan was welcomed by many scientists who had not been happy with Labour's arrangement in which ASTEC reported to the Minister for Science and Consumer Affairs. This much, at least, of Mr Fraser's statement calmed troubled spirits who felt even more secure from the statement that ASTEC "will be a body of the highest status and greatest independence".

It seems to have been only after the election that observers realised that the statement quoted gave no guarantee of independence, except in the way that that term may be defined by a conservative government. Labour was guaranteeing independence for ASTEC by making it a statutory corporation operating under its own Act of Parliament and requiring it to publish its findings. The necessary legislation was, however, one of many important Labour Bills already under debate but which were nullified by the proroguing of Parliament. ASTEC has, in fact, been operating only on an officially "interim" basis.

What happens to ASTEC now is a matter of guesswork. The small organisation has been transferred to the responsibility of the Prime Minister. The Chairman of the Interim body, Dr J. A. L. Matheson, has left his job as Vice-Chancellor of Monash University in anticipation of early confirmation as full-time Chairman. At the time of writing, if the government is acting on the matter at all, it is doing so with sealed lips. The longer the delay, the greater the speculation that "greatest independence" may mean becoming a box in the organisational chart of the Prime Minister's Department or the Department of Science and being treated as a kind of bureau.

### Science department curtailed

No group in the public service would probably be happier with a diminution of ASTEC's independence than the Department of Science, which had never been enthusiastic about ASTEC as a rival in the advisory stakes to government. The department, however, lost much and gained nothing in terms of responsibilities after the election. For a few brief months of potential glory in 1975, the department had



Photos: AIS

James Webster (top) and Clyde Cameron

taken on an extra function of considerable political importance. For some time previously, Mr William Morrison had paved the way for his department to fly the popular flag of consumerism. On taking over Mr Morrison's portfolio, Mr Clyde Cameron was presented with the grander title of Minister for Science and Consumer Affairs. Mr Cameron made a rather ungainly start; his set-piece speeches cast grapes at many targets and displayed little sympathy with his new responsibilities. But, to his credit, he soon settled down to becoming a convinced champion of science *per se*, which Mr Morrison had never done.

In the consumer field, he secured a promise of services from Mr Ralph Nader. Substantial legislative powers were being developed by the new Consumer Protection Division of the department which had been given responsibility of the consumer sections of the forceful Trade Practices Act. In a trade-off with the Attorney General's Department which had thereby lost part of the administration of this Act, the Science Department handed over its Patents operations. Earlier in 1975, the Science Department had established a Consumer Standards Branch. In Mr Cameron's short reign, then, his department enjoyed both a monitoring and prosecuting function in the interests of consumers. This useful union



of powers never enjoyed active consummation; if it had, there is little doubt that it was a potential vote-winner for Labour.

In the December shuffle of responsibilities by Mr Fraser, all consumer activities were removed from Science and split between Attorney General's (consumer protection) and a new Department of Business and Consumer Affairs (consumer standards) where it will require devilish determination to prevent a conflict of interest between business and consumers, to the likely detriment of the latter. Patents, incidentally, continue to bounce around; their administration has now gone from Attorney General's to Business and Consumer Affairs. The fate of consumerism under the Liberals mirrors that of environmentalism which has similarly been buried within a large department of diverse interests, namely Environment, Housing and Community Affairs.

Also after the election, the Department of Minerals and Energy was renamed National Resources under the National Country Party Leader and Deputy Prime Minister, Mr Doug Anthony, as Minister. Professor Messel and Sir Lenox Hewitt retain influence in the department's area of responsibility through the Australian Atomic Energy Commission (AAEC), of which Sir Lenox is Vice-Chairman. Two of the five places on the AAEC have been vacant for some time. Mr Connor was probably keeping his empire-building options open; one of these options was thought to be the creation of a new multi-energy research and development authority which would have incorporated the AAEC, the Bureau of Mineral Resources and the Mineral Research Laboratories of CSIRO, all of which were then under Mr Connor's control. Mr Anthony has not held on to the CSIRO bit, and has shown no indication of how he will treat the AAEC.

The disciplined control on information within the Fraser ministry meant that it was not until some time after the election that the most senior people in CSIRO could obtain clarification of the line of responsibility of their organisation. The new Science Minister, Senator Webster, is now solely responsible for the Science and Industry Act which gives CSIRO its statutory independence. The Minister-CSIRO line is direct and separate from the Minister-Department line. In the face of diminished duties, the Science Department is not out of the woods yet; it will have to work hard to maintain its position which could be called into question whenever Mr Fraser reshuffles his Cabinet or when its Secretary reaches retiring age within a couple of years.

Senator Webster himself is somewhat of an unknown quantity. Before his elevation to the ministry, he was known only to the public through having survived a legal challenge to his eligibility to sit in Parliament on grounds of alleged improprieties in dealings between a family company and the government. Since coming to office, Senator Webster has made only the odd routine announcement on behalf of the organisations under him. He is not yet known for having a personal interest in science. However green a minister he may appear at the moment to senior Australian scientists, he is no different in pre-knowledge of scientific affairs from either of his Labour predecessors, and given his parliamentary reputation as a diligent worker on committees, Senator Webster should make something of his job in a stable political environment.

#### Labour's heritage

If nothing else, the political awakening

of Australia under Labour has produced a quality of information and debate about science policy which is likely to work to the benefit of Australian science in the long run. On a more universal plane, the thoughtful, informed and voluminous reports issued recently, and the subsequent arguments about the very necessity for a Department of Science in any national government, are worthy of separate analysis outside the election context, for the factors involved for science planning may well prove to have global validity.

On the Australian scene, after five years of almost stand-still operations while awaiting times of enlightenment in science policy, it is sad to reflect that the arrival of these very times has coincided with a period of tight-fisted, inflation-dominated management of the economy. Australian scientists had deserved a better deal from both parties; but perhaps they were simply too nice and soft for too long. □

### Ring the changes

Australian science did not escape the impact of the country's political hiatus, as shown by this summary, covering in order the changes over the period beginning a few days before the election:

- The Science Task Force of the Royal Commission on Australian Government Administration recommended abolition of the Department of Science, and redistribution of its functions among other departments.
- Mr Fraser, the second former Minister for Education and Science in a Liberal Government to become Prime Minister (the first was Mr John Gorton), won an outstanding victory, helped by a long-standing imbalance in size of electorates in favour of the conservative parties—Labour received 43% of votes but gained only 25% of seats.
- The first Labour Minister for Science, Mr Bill Morrison (later Minister for Defence) lost his seat by a handful of votes. The second, and last Labour Minister for Science (and Consumer Affairs), Mr Clyde Cameron, retained his seat and immediately lambasted Mr Whitlam for his leadership (which had led to the demotion of Mr Cameron to the Science portfolio).
- Mr Fraser appointed Senator James Webster, of the National Country Party, as Minister for Science, the portfolio which has the lowest seniority. The Department of Science remained in existence, but without consumer affairs.
- The Department of the Environment, another Labour-initiated de-

partment, was abolished and its staff absorbed and reorganised under the umbrella of the new Department of Environment, Housing and Community Development. This department also embraced the Labour-established Department of Urban and Regional Development. Its Minister is Senator Ivor Greenwood, a hard-line conservative.

- The Secretary of the Department of Science, Sir Hugh Ennor, publicly castigated the Task Force's recommendation that his department be abolished, saying it "lacked credibility" and "questionable logic".
- The Australian Academy of Science was publicly drawn into the debate about the usefulness of the Department of Science through the release of its submission to the Task Force. In appealing with feeling for stability in organisation and financing of science, the Academy concentrated on strengthening the advisory roles to government of the Academy itself and the Australian Science and Technology Council (ASTEC); the Academy mentioned the Department only in passing.
- The CSIRO was returned from a dual responsibility to Labour's Minister for Science and Consumer Affairs and Minister for Minerals and Energy to a single Minister for Science under the Liberals.
- The election by staff of a member of the CSIRO Executive, a mild attempt by Labour at worker participation, was stopped in mid-flight just before nominations closed. Official reason—to give time for re-examination of the implications.



## BRITAIN

# 'Cuts' both ways

*The UK Government last week unveiled its White Paper on Public Expenditure and dismayed practically everyone. Colin Norman looks at the implications for British science*

GRADUAL erosion of government support for so-called big science, such as high energy physics and astronomy, little real growth in other areas of basic research, and a virtual freeze on university appointments. Those gloomy prospects are held out in the White Paper which sets out tentative plans for public expenditure for the next five years, and it represents a painful re-ordering of some of the Labour government's most cherished domestic priorities; it has provoked howls of protest from various sources, including the resignation of a junior minister.

The basic strategy is a desperate attempt to curb the inexorable growth in public expenditure by cutting back heavily in such sensitive areas as education and social services as well as defence. The 'cuts' themselves, which are only being made in the expenditure planned for the next few years and thus aim to prevent the growth in spending which would otherwise have occurred, are designed to have their greatest impact in a couple of year's time: the hope is that their effect on unemployment will be mitigated by the fact that worldwide economic recovery should have filtered through to Britain by then. The proposals should not be taken as Gospel, since they will be reviewed each year and they are unlikely to be put into effect if economic recovery doesn't materialise. But the trends are important, and the dismal state of Britain's economy now seems to make it imperative that some blood will be spilled by the Treasury's knife. For the past couple of years, the government has tried to stave off unemployment by spending heavily on public programmes, with the result that with inflation it has run up a massive national debt, for which it is now forced to make staggering interest payments that could outweigh the savings themselves. Unless the debt and the interest payments can be reduced, politically unacceptable tax increases will be inevitable.

## Science and technology

As far as science and technology are concerned, the government is proposing to hold support for basic research constant until the end of the decade. The amount of money flowing to the research councils would increase mar-

ginally this year and next, and thereafter it would decline slightly. Within those totals, however, there is a very deliberate trend away from support of large, capital-intensive programmes, which means that the Science Research Council (SRC) is in for a lean time.

The White Paper itself states that the government intends "to reduce expenditure in the areas of 'big science' (high energy physics, astronomy and space science) supported by the Science Research Council, in order to sustain the other sciences (including applied science) supported by that council, and to enable the Agricultural, Medical, Natural Environment, and Social Science Research Councils to continue to develop programmes based on social need as well as scientific opportunity."

That policy was laid down about a year ago, when the Advisory Board for the Research Councils, an advisory body which recommends how the science pie should be divided among the research councils, suggested that Britain's limited scientific resources should be used to support more people by giving priority to less capital intensive efforts. The result was that SRC's budget was cut by about 2% in 1975, and a similar reduction is expected for each year until 1980.

The axe will fall most heavily on high energy physics, with two accelerators in jeopardy. The first will be the 5 GeV accelerator, known as NINA, which is located at Daresbury, near Manchester. It is due to cease operation at the end of 1977. The other is NIMROD, located at the Rutherford Laboratory, support for which, according to SRC sources, is likely to be reduced although no execution date has been settled.

As for space science, officials in the SRC suggest that British contributions to the European Space Agency will be reduced in the next few years, but the details have yet to be worked out. And the prospect for astronomy is that

there will be no large new capital projects in the next five years.

The other research councils are likely to fare rather better than SRC. The Medical Research Council's funds will grow this year by about 1.7%, those for the Agricultural Research Council are expected to grow by about 2%, and the others are likely to expand in similar fashion.

## Additional impact

But there are other proposals in the White Paper which could affect the science budget. Proposed support for higher education has been greatly reduced from previous plans. The target now is for a total student population of some 600,000 in institutions of higher education by 1981; until last week, the target was 640,000. One impact of the reduction, according to the White Paper, will be that "there will be little, if any, scope for increasing total staff numbers after 1976-77." That stagnation in university hiring would follow on equally tight period in academic appointments.

Another dismal aspect of the proposals for education is that "capital expenditure on new buildings, . . . will continue to be severely restricted and more intensive use of available premises will accordingly be required". Failure to commit adequate funds to university facilities is indirectly likely to hurt the research councils. There is some fear that the research councils will find themselves paying for equipment and facilities which would normally come from general higher education support funds. Again, the SRC would suffer most.

In another area earmarked for the Treasury's knife, defence research and development, few details have yet been released. But last week Defence Secretary Mr Roy Mason announced that "we are reviewing the future levels of research and development on defence against chemical and biological warfare carried out at the Porton research establishments, with the object of making significant economies". □

Science Budget expenditure analysed by main forms of research and training support (in £ million)

	ARC	MRC	NERC	SRC	SSRC	Total
Research grants and contracts	1.1	8.3	2.2	19.7	3.0	34.3
Research units	1.2	10.6	0.0	0.0	0.5	12.3
Research Council Establishments	3.5	5.9	12.3	34.7	0.0	56.4
Research Council grant-aided institutes	6.7	0.0	1.3	0.0	0.0	8.0
Postgraduate Awards	0.1	1.9	1.5	10.1	3.9	17.5
International subscriptions	0.0	0.6	0.0	24.7	0.2	25.5
Centrally supported schemes and administration	0.5	1.6	1.9	7.2	1.1	12.3
	13.1	28.9	19.2	96.4	8.7	166.3

Expenditure based on 1975-76 figures, excluding expenditure by the Natural History Museum and the Royal Society (£4.6 million).

## EMBO

## Transatlantic translation

*A unique proposal for regulating genetic research in Europe has been developed. Colin Norman reports*

A MECHANISM for controlling potentially hazardous genetic manipulation experiments in Europe will soon be proposed by the European Molecular Biology Organisation (EMBO). A special EMBO committee, consisting of 10 leading European biologists, agreed at a meeting last week that regulations already drafted to control such research in the United States are more than strict enough to protect public health, and a statement expected next week will recommend that they should be implemented in Europe.

Although EMBO is purely an advisory body, with no authority to regulate research, its recommendations will probably carry considerable weight. Its proposals are especially relevant for smaller countries which are unlikely to establish their own mechanisms for controlling genetic manipulation experiments, and, if implemented, they would set up a unique arrangement for regulating scientific research on an international basis.

The experiments involve the use of

newly discovered enzymes to transplant genes from virtually any living organism into viruses or bacteria, and the proposed US regulations, drafted last December but not yet finalised, would outlaw some of the more hazardous experiments and require that others be performed under special safety conditions. In particular, they specify that several types of experiments should only be carried out by transplanting genes into bacteria or viruses which have been genetically crippled to render them incapable of surviving outside an artificial laboratory environment.

Some members of the EMBO committee considered those regulations stricter than necessary to protect public health, but agreed that not all European countries should repeat the tortuous process of drafting their own regulations. So, with the proviso that the US controls are not made yet more stringent, the committee recommends that each country should establish its own national committee to vet applications for research grants involving gene transplant experiments and decide whether they conform to the US regulations. EMBO also suggests that an international committee, possibly under the auspices of the European

Science Foundation, should deal with problem cases.

One possible problem area involves the certification of crippled strains of bacteria or viruses. The US regulations detail the criteria by which modified microorganisms should be judged sufficiently crippled for use in gene transplant experiments, and one strain of bacterium recently produced at the University of Alabama is expected to meet the criteria. Although strains produced in the USA will be made available throughout the world, other modified strains may be produced in Europe.

National committees would be responsible for certifying whether or not such strains meet the criteria, but the EMBO committee is willing to offer its advice in specific instances. It has also offered to advise European governments and individual scientists about technical aspects of genetic manipulation research and safety precautions, a service that could be particularly useful for countries with small numbers of researchers.

While some European countries, including Britain, are already developing their own regulations and are unlikely to adopt the US version lock, stock and barrel, the idea of an international committee to discuss problem cases is likely to have considerable support throughout Europe. □

## SOVIET JEWRY

## Tailoring tactics to suit

*Vera Rich reports from Brussels, where the Second World Conference of Jewish Communities on Soviet Jewry was held last week.*

THE problem of Jewish scientists and other academics wishing to emigrate from the USSR to Israel continues to arouse debate. So too does the degree to which scientists should commit themselves to helping and campaigning for their disadvantaged colleagues. At the conference on World Jewry, a Special Commission on Science and the Humanities, chaired by Professor Yuval Neeman of the Israel Committee for Soviet Jewry, resolved to set up an International Federation of Concerned Scientists. Professor Denis Schiame of Oxford explained that its purpose would be to gather and disseminate information and to coordinate the activities of its affiliates from all countries where committees already exist or are being formed.

But the precise policy to be followed provoked lively discussion. A large

contingent, headed by the Nobel Prize-winner for Physics Dr Polykarp Kusch, stressed the importance of using international scientific cooperation agreements, which at present tend to favour the USSR, to exert pressure on behalf of disadvantaged scientists. The case of *refusnik* Aleksandr Lerner and the Fourth International Conference on Artificial Intelligence was cited: his attendance and active participation was secured after pressure was applied that threatened to change the venue from Tbilisi to a location outside the Soviet Union. But Dr Gabor Dessau of Pisa stressed the beneficial effect which foreign contacts could have on Soviet scientists who, he said, are often themselves secretly in favour of a greater freedom of movement. The idea of a total boycott of Soviet science was ultimately firmly rejected. It was agreed that the tactics to be used should rather be tailored to each specific problem as it arose.

Golda Meir referred to the "education tax" on Soviet academics, and cast doubt on the possibility of a mass

emigration of scientists seriously depleting the Soviet Union: dismissal was the immediate consequence of a visa application, she argued, so that "their brains are drained already". Aleksandr and Evgenii Levich, referring to the problem of classified information, recalled that their father, Academician Venyamin Levich, had been promised an exit visa at the end of 1975, when his knowledge would no longer be "classified", this promise was later withdrawn.

Mention was also made of Soviet delegations to international conferences which regularly consisted of "representatives" chosen for their standing with the authorities and not their scientific knowledge or relevance.

The absorption of scientists from the Soviet Union into the Israeli economy, although not a direct concern of the conference, was discussed in various formal and informal gatherings. The current policy, said Dr. Eliezer Rafaeli, Rector of the University of Haifa, is to absorb them into existing institutions, establishing new departments or laboratories if necessary, but not setting up new universities. □

## MEDITERRANEAN

## Treaty in store

*Sixteen Mediterranean states meeting in Barcelona earlier this month adopted a treaty on the protection of the Mediterranean against pollution which augurs well for a future "action plan". Robert Allen reports*

AN interval of only two years separates discussion of the first principles of the Mediterranean anti-pollution treaty and its signature. There are two main secrets of this success, which comes despite the sharp political differences of the participants (Spain, France, Monaco, Italy, Malta, Yugoslavia, Greece, Turkey, Cyprus, Syria, Lebanon, Israel, Egypt, Libya, Tunisia and Morocco).

One reason is the existence of a regional scientific and resource management body, the FAO's General Fisheries Council for the Mediterranean (GFCM). Arabs and Israelis, Greeks and Turks, have worked together on the Council for some years now, and are used to each other at the scientific and technical levels of government. Secondly, there is the United Nations Environment Programme (UNEP), which can not only bring governments together, but can locate, harness and coordinate legal, scientific and planning expertise scattered amongst numerous research institutes throughout the Mediterranean area and within the UN's many (often competing) specialised agencies. It is this capability which is the key to an ambitious "action plan" being developed by UNEP and the Mediterranean coastal states and involving co-operative action on four related fronts: scientific, legislative, institutional, and planning.

UNEP has set up seven research projects\*, to run initially for two years, in order to establish a basic pollution profile of the Mediterranean. Each project will employ a network of laboratories, coordinated by UNEP, appropriate specialised agencies and a designated national research centre. So far, eight countries (Algeria, Cyprus, Israel, Italy, Lebanon, Malta, Spain and Yugoslavia) have nominated a total of 19 laboratories. Other countries are expected to nominate laboratories soon.

Where necessary, UNEP will provide

analytical equipment and training in its use. It will also fund a common maintenance service to avoid long gaps in coordinated data collection. The International Atomic Energy Agency's Marine Radiation Laboratory in Monaco will help ensure compatibility of results by setting monitoring standards and providing intercalibration services.

The entire scientific programme is expected to cost some \$10 million (almost \$2 million from UNEP, and about \$8 million from participating governments). For this, the coastal states of the Mediterranean will obtain a reliable pollution profile of the marine environment, derived from simultaneous measurements of pollutant levels in the same taxa, at a large number of sampling points, with the same instrumentation. There will be a comprehensive range of strictly comparable national data from the entire region instead of the present hodgepodge of hard facts, circumstantial evidence and anecdote. Without this data, planning the rational exploitation of the Mediterranean commons would be virtually impossible. It would also be extremely difficult to maintain the present rapid legislative progress when more contentious issues are tackled.

The treaty consists of a convention supported by two protocols (one on dumping, the other on cooperation in the event of pollution emergencies, such as oil spills), but the next one to come, controlling pollution from land-based sources like rivers and coastal outfalls, is the one most likely to founder if the data base is insecure. Mediterranean nations will be confronted with the substantial economic differences between north and south and difficult trade-offs between one sector of a national economy and another. With non-Mediterranean up-river states also involved, a reliable pollution profile will be essential.

Much of the additional promise of the action plan lies in its integrated planning effort, which aims to help governments develop the shared resources of the Mediterranean in a sustainable fashion. Permanent channels for co-operative planning are being set up, based on a conception of the Mediterranean Sea area as an ecological unit. It sounds slightly over ambitious, but as Peter Thatcher, Director of UNEP's Geneva office, remarked, the adoption of the treaty demonstrates that the "political leaders of the Mediterranean have overcome the divisive issues of today in order collectively to preserve their common heritage and discharge their responsibilities to future generations". □

## CANADA

## New breed

*Canada's space programme was back on the nation's front page and television screens again briefly last month when the United States launched her neighbour's latest satellite, the CTS (communications technology satellite). David Spurgeon reports from Ottawa*

CANADA'S space programme has been one of her most successful scientific and technological ventures. Alouette I, the first satellite designed and built by a nation other than the United States or the Soviet Union, was still sending back useful ionospheric data 10 years after its launch in 1962. Three other Canadian made scientific satellites successfully followed that (Alouette II, ISIS-I, ISIS-II), and two domestic communications satellites (Anik-I and Anik-II) both continue to serve the country's communications needs, the former being the first such satellite to be used in a geostationary orbit.

Canada's vast distances, severe climate and scattered population have all meant that its space program has been oriented toward electronic methods of communication. The new CTS satellite follows this pattern. It is considered the forerunner of a new breed of high-powered communications satellites that will be able to transmit directly to small, low-cost earth stations sometimes in remote and otherwise inaccessible locations.

The CTS is in fact the most powerful communications satellite now operating, by virtue of three major subsystems. One of these is the travelling wave tube amplifier: its power output of 200 watts operating at an efficiency of 50% compares with the six watts at 30% efficiency of the current generation of tubes used in communications satellites. A pair of lightweight, extendable antenna arrays carries enough solar cells to provide one kilowatt of power to the satellite; and a three-axis stabilization system, employing a fixed momentum wheel and hydrazine gas thrusters, maintains the aiming accuracy of the antenna and keeps the spacecraft oriented toward the sun (rather than letting the spacecraft spin as is usual) to ensure full use of solar power.

The CTS satellite is able to employ high power transmitters because it operates in a new frequency band of 12-14 gigahertz allocated for broadcast satellites. Current systems operate in the 4-6 gigahertz band, which is shared by other types of system on the ground. For this reason, satellite power levels must be limited to prevent interference

\*The 7 projects include: (1) baseline studies and monitoring of oil and petroleum hydrocarbons in marine waters (coordinated by UNEP and the Intergovernmental Oceanographic Commission, IOC, of UNESCO), (2) baseline studies and monitoring of metals, particularly mercury and cadmium, in marine organisms, and (3) of DDT and other chlorinated hydrocarbons in marine organisms—both coordinated by UNEP and the GFCM.

## USA

with ground systems: previously this was a major factor dictating the use of large and expensive ground antennas.

Antenna diameters for the CTS range from 32 inches to 30 feet. The only other satellite designed to transmit high-quality colour television to small, simple ground stations is the ATS-6 (applications technology satellite), which was launched in 1974 and is now being used by the government of India to relay educational programs to isolated villages. The ATS-6 must use a 30-foot antenna, and its effective radiated power is 53 DBW (decibel watts), whereas CTS's is 59 DBW.

Canadian groups have designed 26 experiments for the CTS, to begin in May. They include:

- One-way video and two-way audio transmission to provide continuing medical education for doctors in remote areas.
- High-quality telephone links to and from remote camp-sites in the James Bay area, where a power dam is being built.
- Curriculum-sharing between Carleton University, Ottawa, and Stanford University, California, using a digital video compression technique.
- Provision of computer-communications networks for native peoples in Canada's isolated northern regions, and evaluation of the potential.
- Linking of two francophone communities in different parts of Canada—Zenon Park, in Northern Saskatchewan, and a community in Quebec—for interchange of cultural and educational programs via two-way audio and video links. Other projects involve propagation measurements, a system for sharing telephone channels, and a fast frequency-shift keying system for high-speed digital data transmission.

The Canadian Broadcasting Corporation will try to demonstrate direct television reception from the satellite using simple, domestic-type equipment from laboratories in Japan and perhaps Europe. The satellite is also to be used for remote coverage of the 1976 Olympic Games equestrian competition at Bromont, Quebec. The signals would help in assessment of portable ground stations and reception and transmission from large, populated areas.

The United States and Canada will share equally in experiment time during the satellite's lifetime, using it on alternate days. If the experiments go as planned, the remote areas of many parts of the world—as well as Canada and the United States—may soon benefit from a multitude of communications services presently not economically feasible using conventional ground-based methods. □

With only one dissenting voice—that of Senator John V. Tunney of California—a subcommittee of the Joint Committee on Atomic Energy has produced a report giving full support to the liquid metal fast breeder reactor (LMFBR) programme and suggesting that “the time has come to end the discussion over whether or not [the United States] should have a breeder research and development program. Rather, national attention should be turned toward solving the outstanding problems associated with the program and its eventual commercialisation.”

Senator Tunney identifies what he describes as a failure to deal adequately with many fundamental issues and questions surrounding the breeder programme, and in particular he objects to the subcommittee's attempts to see discussion of the programme stifled. That would be an abdication of responsibility, he says, as well as inconsistent with the subcommittee's conclusion that the building of the Clinch River Breeder Reactor in Tennessee would not represent “an irreversible commitment to commercialisation.” (The Clinch River reactor, a 350-MW demonstration plant, is at present expected to be completed in 1983, at a cost of about \$2,000 million.)

The subcommittee, chaired by Congressman Mike McCormack of Washington, heard four main objections from opponents of the breeder reactor programme. It was argued that development costs would turn out to be prohibitive, that estimates by the Energy Research and Development Administration (ERDA) of indigenous uranium resources were too conservative, that energy demand would not grow as fast as projected, and that problems like plutonium toxicity and waste management would prove to be insoluble.

The subcommittee brushes those objections aside. One of its chief arguments in support of the LMFBR programme, for example, hinges on the suggestion that conventional reactors could start to run out of domestic supplies of uranium early in the twenty-first century, and unless the plutonium-producing capability of the breeder reactor is developed, the nuclear industry could grind to a halt. ERDA estimates, in fact, that there are about 3.6 million tons of uranium, available at a cost of less than \$30 per pound, in the United States. That is sufficient to guarantee a lifetime's fuel only to those reactors expected to be built before the mid-1990s.

● After 18 months of uncertainty, the future of Sacramento Peak Solar Observatory in New Mexico seems assured now that the National Science

Foundation (NSF) has agreed to provide \$1.45 million “to maintain the observatory at a productive level”. The US Air Force, which has operated the observatory since 1952, will, however, still maintain a presence, and provide some of that money—\$700,000 in the first year of the new arrangements, and \$450,000 in the second. After that it is not clear what, if any, contribution it will make, or how the NSF will make up the shortfall.

Even with the Air Force chipping in a further \$250,000 for the salaries of those remaining and \$250,000 in grants, the observatory will, according to its acting Director, Dr Richard Dunn, have to sustain cuts of 15% in its present budget of \$2.2 million and 27% in its staff of 63, as from June 30.

The new funding scheme was hammered out by a committee chaired by Professor Martin Schwarzschild of Princeton University, and the NSF has now formally accepted it. Agencies such as NASA and ERDA were also involved in the discussions: NASA already has a group working at Sacramento Peak, and the observatory's research programme contains sufficient plasma physics, hydrodynamics and so on for ERDA to find it relevant to its own fusion research activities. (In fact at one time it seemed as if ERDA might pick up the tab for the observatory itself.) As the Air Force's contribution diminishes, the NSF will undoubtedly be looking to other agencies to stump up some of the extra money needed. The matter awaiting resolution now is precisely how the observatory will be managed once the Air Force relinquishes its overall responsibility.

● The fifth annual report to Congress on marijuana and health shows that more than half of those between 18 and 25 have used the drug at some time, and that “what was once clearly statistically deviant behaviour has become the norm for this age group.” The report also says that the correlation with level of education has now all but disappeared. The authors of the report, produced by the Department of Health, Education and Welfare, say, however, that the drug's long term effects are not yet properly understood. Another aspect of the drug's use seems to have altered: whereas users of marijuana were once less likely than non-users to consume alcohol, they now seem more likely to do so. “Frequently the two drugs are used simultaneously,” says the report, “a combination that may be more hazardous than either used alone”.

**Roger Woodham**  
Washington



## IN BRIEF

**Soviet treaty call**

The Soviet Union called for an early treaty covering the development of weapons "still more pernicious" than nuclear arms when the 15th session of the Committee on Disarmament opened in Geneva last week. Delegates from 30 nations, urged to adopt a Soviet draft agreement on the issue, remained in the dark about the precise nature of the doomsday weaponry hinted at, but the Soviet delegate stressed that the reference was not to environmental or weather modification techniques, on which the USSR and USA are expected to approve a draft ban treaty by the end of the 1976 conference. The USSR also renewed its call for a committee of nuclear and non-nuclear states to discuss a comprehensive test ban treaty.

**Nuclear safety**

Three nuclear scientists who recently quit responsible positions with General Electric in a stand against the US nuclear programme last week told a

Joint Congressional Committee that many US nuclear installations should close because they are below Federal safety standards. In Europe, the Nuclear Energy Agency of the Organisation for Economic Cooperation and Development has meanwhile calculated that ambient radiation levels would increase by about 6% if electricity production needs were met entirely by fission reactors—an increase reckoned to pose less overall risk than hazards associated with existing methods of production. News has also come of a similar assessment attempted in the USA 20 years ago when, between 1945 and 1947, the US authorities secretly injected 18 terminally ill people with doses of plutonium equivalent to levels that would normally be received by plutonium workers in 100–800 years. Three of the human guinea pigs are still alive.

**Nuclear arms**

As a safeguard against possible terrorist attempts to seize nuclear fuel in Britain, a Bill presented to Parliament

last week extends the authorisation whereby security forces guarding UK Atomic Energy Authority establishments carry firearms to cover other nuclear installations housing "accessible" nuclear materials and UKAEA police accompanying fissionable material in transit. MPs unhappy about the Bill have already promised action at its second reading.

**EEC fish limits**

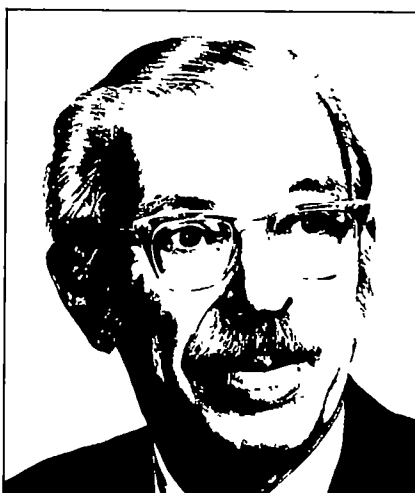
In a move anticipating an extension of international fishing limits during next month's Law of the Sea conference in New York the European Commission has called for the setting up of a 200 mile community limit around EEC states. Under the proposals, presented in Brussels last week, each nation will retain exclusive rights over a 12 mile strip within the wider community zone surrounding its own territory. A scientific and technical committee would advise fisheries officials of the Nine on necessary conservation measures before catch quotas and share-out proportions are drawn up for Community waters.

THE Delaney Clause of the Food Additives Amendment forbids the use of food additives that are carcinogenic, or animal feed additives that are carcinogenic and leave residues in the meat. The bureaucratic axe is being sharpened for bacon. On January 19 this year the FDA banned Red No. 2 (Amaranth), and on January 7 announced a forthcoming ban on diethylstilbestrol (DES) in beef production at an estimated cost of \$503 million annually to consumers. Representative James Delaney, evidently not satisfied with the effectiveness of his clause, introduced special and apparently superfluous legislation (HR 9837) for the latter purpose. Not to be outdone, the National Cancer Institute (NCI) recently warned pregnant women against eating beef liver.

Herbst and co-workers reported in 1975 on "benign alterations of the genital tract", especially abnormal vaginal mucosa, in a series of 110 females who had been exposed *in utero* when their mothers received DES prescribed during pregnancy. No cases of cancer were observed, although vaginal adenosis, which is suspected by some authors of being a "precancerous lesion", occurred in 35% of cases. The absence of cancer fits Herbst's earlier observation that the risk of cancer may be considerably less than 4 cases for 1,000 exposures. However, the dosage of DES received by the pregnant women is of extraordinary interest; it was

increased stepwise from the sixth to the 35th week for a total of 12.3 grams and a daily average of 60 mg per patient.

In other investigations, the "DES" content of liver from implanted cattle

**Delaney's year****THOMAS H. JUKES**

was found to be 0.12 parts per 10<sup>9</sup>, so that 12.3 grams would be present in 100,000 tons of contaminated liver. The total amount of beef liver produced in the USA is about 140,000 tons per year. More than a century of beef production would have been needed to supply DES in beef liver to the women in the study, none of whom produced offspring who deve-

loped cancer. This assumes that the "residues" are actually DES, which is now known not to be the case. No wonder Mr Delaney is impatient for his ban; he may be worried that common sense will take over.

In a speech in the Senate, calling for a ban on DES in beef production, Senator Edward Kennedy said the ban had been requested by Dr Frank Rauscher, Director of NCI, and that public funds of about \$700 million annually were furnished to NCI, which provides scientific guidance to him on questions of cancer. Perhaps a small allocation, say \$100, should be set aside for the purchase of a pocket-size calculator to compute the actual risk from a daily intake of one molecule of DES, which NCI scientists have testified might cause cancer. Part of the \$100 could be used for a textbook on nutrition, so that NCI could learn that beef liver is the best dietary source of folic acid, and that folic acid deficiency is common during pregnancy.

Indeed, folic acid was discovered, as a result of the anaemia in pregnant women caused by its deficiency, by Dr Lucy Wills in 1931. The deficiency is still prevalent throughout the world, as noted by the World Food Congress in Rome, 1973. A continuation of the dietary lack of folic acid in pregnancy will be aided by NCI's recommendation against consuming beef liver. The protective effect against cancer of this recommendation seems dubious.

# news and views

## Receptor maps for viruses

from Arie J. Zuckerman

VIRUSES are not motile and they rely on such processes as diffusion and transport within body fluids to bring them into contact with susceptible cells. The critical step in successful viral infection is the susceptibility of cells, and the means by which viruses are incorporated into host cells has been the subject of much research. The susceptibility of cells is usually determined by early steps in virus-cell interactions which include the attachment and penetration of the virions and the release of the viral nucleic acid in the cells. With some animal viruses such as the influenza viruses and some of the picornaviruses such as Cocksackie virus and poliovirus, attachment is associated with the presence of specific receptors for viral adsorption on the cell surface, but whether receptors are required for all viruses is not known. The presence or absence of receptors on the cell surface depends on several factors which include the particular type of tissue or organ, stage of maturity, changes in the physiological environment such as culture *in vitro*, and genetic factors involving cellular rather than immunological mechanisms.

On the other hand, there are also viruses, including arboviruses, poxviruses and herpes viruses, which will grow in a variety of cell types, and therefore there appears to be little specificity about the adsorption process of such viruses. In these instances a mechanism such as phagocytosis or viropexis may be important in the incorporation of virus into susceptible cells. Another mode of viral entry entails fusion of the viral envelope with the plasma cell membrane. Fusion takes place by a process of alignment of the two membrane structures, followed by the formation of a channel between virus and the cytoplasm through which the nucleocapsid passes into the cell. A more recently described mode of entry of virus into the cell is by a process of membrane fission rather than by viropexis.

Lonberg-Holm and his colleagues point out on page 679 of this issue of

*Nature* that the specific receptors for several nonenveloped viruses are present on cells in limited number and therefore these can be saturated with excess virus. They report results of experiments in which the attachment to HeLa cells of highly purified virus particles radiolabelled with carbon, sulphur and phosphorus was measured. Four receptor families were found. The first family contains receptors to human rhinovirus types 2, 1A and 1B. The second family contains receptors to human rhinovirus type 14 and Cocksackie A21 and a number of other rhinovirus serotypes. The third family contains poliovirus, and the fourth contains Cocksackie B3 and probably the other Cocksackie B serotypes as well as several serotypes of adenovirus. It is reasonable to consider that receptor specificity influences virus tropism in the tissues thereby contributing to the patterns of pathogenesis of virus infections. An example is the finding that Cocksackie A21 shares receptors with a large number of human rhinoviruses and this may be related to the ability of Cocksackie A21 to cause coryza. Another example is that both Cocksackie B viruses and adenoviruses produce persistent infections in HeLa cells cultured in the presence of human serum.

Burrows pointed out, however, (*Microbial Pathogenicity in Man and Animals*, 303-332, Cambridge University Press, 1972) that many investigators have attempted to associate characteristics of virus growth and behaviour *in vitro* with virus behaviour in the intact host. Characteristics such as tissue receptor activity, the production of or susceptibility to interferon, growth and cytopathogenicity in cultured cells and virulence for laboratory animals have all been linked to differences in virulence for the normal host. The determinants of virulence, although clearly related to those of virus growth, differ markedly between viruses and indeed between strains of the same virus. It was Chaproniere and Andrewes (*Virology*, 4, 351; 1957) who

stressed that the ability of many viruses to grow in monolayer cell cultures derived from tissues or hosts which are not susceptible to the virus, limits the usefulness of such cultures for basic studies of susceptibility as they relate to the intact host.

It is generally considered that the morphological and functional capacity of cells maintained in organ culture may offer a system for cultivating viruses which closely simulates conditions in the intact host. Examples of the specificity of the effect on the target organ and differential susceptibility of different hosts are found within each class of viruses and organ cultures have reproduced many, but not all, of the phenomena of specificity observed in the intact animal. Extension of the studies reported by Lonberg-Holm and his colleagues and the precise identification of the properties of the specific virus receptors may provide a key to the understanding of viral pathogenesis and perhaps also to the prophylaxis and even therapy of virus infections. □



### A hundred years ago

After discussing the results obtained by Stoney, Thompson, and Clerk-Maxwell, mainly derived from the properties of gases, I come to the conclusion that in the present state of our knowledge the best approximation that we can make to the size of the ultimate atoms of matter is the mean of their determinations. I adopt for simplicity  $\frac{1}{1000}$ th of an inch as the unit of length, and  $\frac{1}{1000}$ th of an inch cube, or  $\frac{1}{1000000000}$ th of a cubic inch, as the unit of volume. In the case of a true gas the number of atoms in the length of  $\frac{1}{1000}$ th of an inch at 0 °C, and a pressure of one atmosphere, would be 21,770, and hence, in  $\frac{1}{1000}$ th of an inch cube, about 10,320,000,000,000.

From *Nature*, 13 (February 24), 332; 1876.

# Charm: what else could it be?

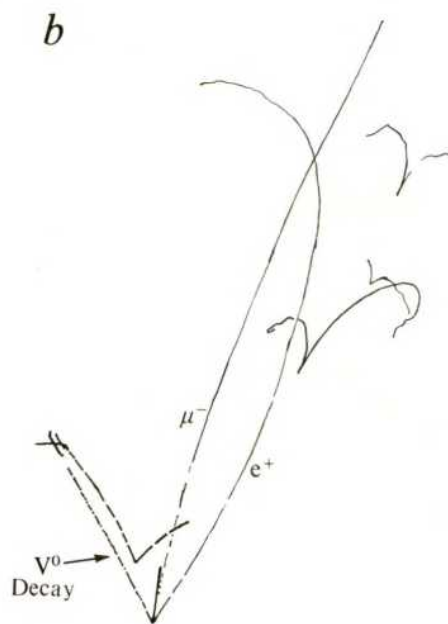
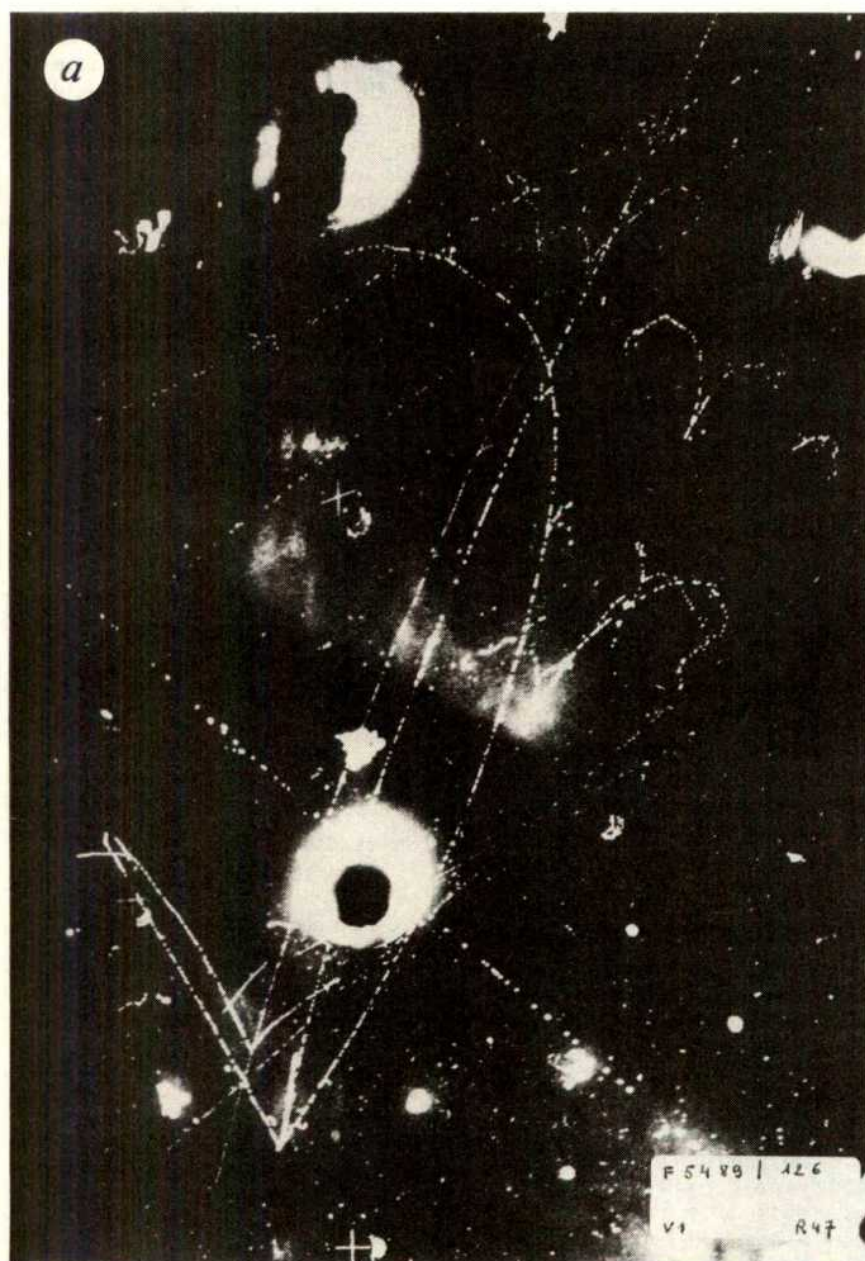
from W. T. Toner

THE peculiar bubble chamber event cautiously put forward by the European "Gargamelle" collaboration as possible evidence for charm now has two companions (Deden *et al.*, *Phys. Lett.*, **58B**, 361; 1975; Bleitschau *et al.*, *Phys. Lett.*, **60B**, 207; 1976), and four similar events have been found in pictures from the fifteen foot diameter bubble chamber in the neutrino beam at the Fermi National Accelerator Laboratory (FNAL) near Chicago. One event could always have been a freak chance but not three, still less seven. A new physical effect has been dis-

covered.

All seven events are of the type shown in Fig. 1, where an unseen neutrino from the beam interacts with a nucleus in the chamber liquid to produce several particles one of which is, as usual, a negative mu-meson. Two of the other particles are uncommon: a positron and a neutral V particle, so called because of the pattern made by the tracks from its decay a few centimetres from the main interaction in which it was produced. The Vs are members of the group of "strange" particles. The positron signals the

weak (radioactive  $\beta^+$ ) decay of some particles whose lifetime must be extremely long on the subnuclear time-scale as otherwise the decay would be strong. But since the positron appears to come directly from the interaction point the decay must take place within a millimetre and the lifetime must therefore be significantly less than that of the Vs. No known particle has a lifetime in this range although some of the hypothetical charmed particles should have, and should frequently involve strange particles in their production and decay.



**Fig. 1** *a*, One of a stereoscopic set of photographs of an event in the Gargamelle bubble chamber at CERN. Only charged particles leave tracks. The neutrino beam enters from the bottom of the picture. *b*, Sketch of the tracks involved in the event. (Stereoscopic reconstruction eliminates the others.) The mu-meson ( $\mu^-$ ) leaves the chamber without interacting; the positron ( $e^+$ ) has a rapidly changing curvature and is associated with electron-positron pairs; the  $V^0$  itself leaves no track, what is seen are the two tracks from its decay. The other two particles emitted in the primary interaction are unimportant to the interpretation.



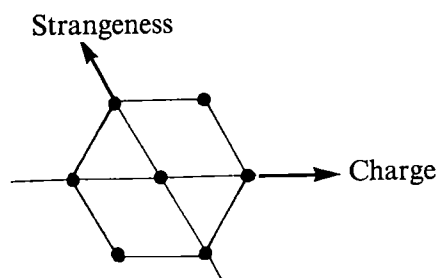


Fig. 2 A two-dimensional pattern in charge-strangeness space. There are three particles at the origin and one at each of the six corners.

What is charm or for that matter, strangeness? Both are properties akin to electric charge which are supposed to be possessed by elementary particles in quantities of  $0, \pm 1 \dots$  units. Everyday particles like the proton and neutron have charm and strangeness zero. Like electric charge, the total amounts of charm and strangeness must be conserved in all strong (nuclear) or electromagnetic interactions but unlike electric charge, neither need be conserved in  $\beta$ -decay or other weak processes.

Strangeness has been an accepted fact for more than 20 years. The concept was invented in 1952 by Pais (*Phys. Rev.* **86**, 663; 1952) to explain why the V-particles discovered by Rochester and Butler (*Nature*, **160**, 855; 1947) should be copiously produced and yet decay only slowly. Copious, strong, production of pairs having opposite and mutually cancelling strangeness would be allowed; but all particles die alone, and the metamorphosis from strange back into ordinary matter could take place only slowly, by weak decay.

The idea was enormously successful and is essential to the  $SU_3$  or Quark classification scheme which fits all particles known up to a year ago into triangular and hexagonal patterns such as that in Fig. 2 and which interrelates their properties in like manner. Electric charge is one axis of the pattern and strangeness the other. Charmed particle production and decay would obey similar rules and charm would add a third dimension to the patterns, as in Fig. 3. The most obvious experimental consequence would be the existence of the many new particles in the third dimension, with charm  $\pm 1, \pm 2$ , whose decays into uncharmed matter would be weak, and of a few new particles composed of pairs of oppositely charmed quarks with nett charm zero. These might or might not have long lifetimes.

In the case of charm, the theory came before the experimental evidence. It was invented by Bjorken and Glashow in the spirit of exploring the consequences of adding a new dimension to the patterns (*Phys. Lett.*, **11**, 255; 1964). Charm remained a theoretical toy which predicted too many new effects until the Gargamelle collaboration found another new class of neutrino interactions in which the neutrino did not turn into a charged mu meson as usual, but retained its identity (Hasert *et al.*, *Phys. Lett.*, **46B**, 138; 1973). It was difficult to reconcile this observation with the known absence of equivalent processes among strange particles without invoking charm and the elegantly constructed cancellation mechanism of Glashow, Iliopoulos and Maiani (*Phys. Rev.* **D2**, 1285; 1970) which linked charm with strangeness. A consequence was that this link should show up by the frequent appearance of strange particles in charm non-conserving weak decays and in the weak production of single charmed particles by neutrinos.

In the summer of 1974, charm was an exciting theoretical conjecture. Enthusiasts, such as Iliopoulos, were willing to wager a case of wine on its imminent discovery (*Proc. XVII Int. Conf. on High Energy Physics*, (edit. by Smith, J. R.) III-100 SRC, 1974), though others remained sceptical. A few months later the  $\psi$  (or J) particles were discovered and found to have many of the properties forecast for the charm = 0 or "hidden charm" members of the family. Although alternative explanations for the  $\psi$ s were and are still tenable it seemed to most that definite proof of the existence of charm was very near. That seems even more surely the case now, but the last stages of the search have proved difficult.

The only direct evidence other than the  $\psi$ s has come from experiments with neutrinos, which interact so rarely that their mean free path is hundreds of Earth diameters. Any apparatus designed to observe large numbers of interactions is necessarily massive, and is clumsy in even the most skilled hands. A group using a detector weighing several hundred tons composed of iron slabs interleaved with scintillation counters and spark chambers reported two "di-muon" events at the 1974 conference (see C. Rubbia, *loc. cit.* IV-119) and were later able to show with many more events that weak decays were taking place among the products of neutrino interactions at a rate which could not be explained by any known particle (Benvenuti *et al.*, *Phys. Rev. Lett.* **35**, 1199; 1975). But although many events had been seen and the effect was by then undisputed,

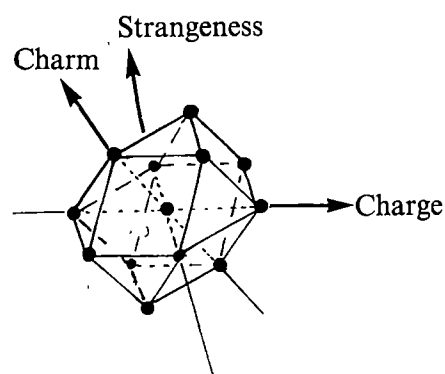


Fig. 3 A three-dimensional pattern in charge-strangeness-charm space. There are four particles in the centre, at the origin, and one at each of the twelve corners.

charm was a very plausible rather than a necessary interpretation.

The new bubble chamber events clearly establish the expected link with strangeness and set loose bounds on the lifetime of the new particles but even their graphic detail is insufficient to determine the precise nature of the interaction because in each event there are two neutrinos whose energies are unknown: the one which initiated the event and a second one emitted in an unknown direction from the  $\beta$ -decay. Without mass values and decay schemes one cannot begin to construct the pattern to see if it fits with charm, let alone make the sophisticated tests needed to prove that it solves the theorists' problems.

The next step is to find cases where the decay does not involve a second neutrino in order to establish the masses of the supposed charmed particles. Events of this type will be harder to identify because they will also lack the positron which singles out the weak decay. One such event with other strikingly characteristic features of charm has been reported (Cazzoli *et al.*, *Phys. Rev. Lett.* **34**, 1125; 1975) but subsequent experiments with greater sensitivity have failed to reveal others of the same kind. It may be necessary to collect many events with less obvious distinguishing features and search in the mass distributions of different combinations of supposed decay products for narrow peaks, which would be alternative evidence for the existence of particles with unusually long lifetimes. This is a sure and well-timed method of finding new subnuclear states although it takes time and patience. Meanwhile, the commonest answer to the question what else it could be is: something else very like charm.  $\square$



## Bacterial cytochromes *c* structure

from D. R. Thatcher

COMPARISONS of protein structure at the three-dimensional level are able to distinguish similarities, and therefore probable evolutionary connections, which would not be convincing at the amino acid sequence level. The best example of the application of this approach is the discovery of the characteristic super-secondary structure of enzymes which bind dinucleotides. Now that the X-ray crystal structure of four different types of cytochromes *c* is known, a detailed three-dimensional comparison has been made (Dickerson, Timovich and Almasy, *J. molec. Biol.*, **100**, 473; 1976), and the evolutionary implications discussed.

Eukaryotic cytochromes *c* form a clearly homologous group of proteins, both at the three-dimensional level (horse, bonito and tuna crystal structures are available and show an almost identical folding pattern) and at the amino acid sequence level (sequences from over 70 different eukaryotes have similar lengths and have a minimum of 25% of their sequence identical). By contrast bacterial cytochromes *c* show a formidable variation in chemical structure and physical properties; a reflection of the extreme antiquity and diverse modes of energy metabolism in this group of organisms. Dickerson *et al.* have compared the already published three-dimensional structures of tuna cytochrome *c* and *Rhodospirillum rubrum* cytochrome *c*<sub>2</sub> (a purple non-sulphur bacterium) with the new structures of cytochrome *c*<sub>550</sub> from *Paracoccus denitrificans* and *Pseudomonas aeruginosa* cytochrome *c*<sub>551</sub>.

All four cytochromes differ significantly in the length of their polypeptide chains (cytochrome *c*=103 residues; *c*<sub>2</sub>=112 residues; *c*<sub>550</sub>=134 residues and *c*<sub>551</sub>=82 residues).

Although the variation in amino acid sequence between these cytochromes is large (in the case of *c*<sub>551</sub> primary structural similarity can barely be discerned), the overall folding pattern around the haem prosthetic group is recognisable in all four cytochromes. The bacterial cytochromes mainly differ from the tuna cytochrome *c* by additional residues in the external loops around positions 53 and 75 (cytochrome *c* numbering) whilst *c*<sub>550</sub> also has an insertion in a loop in the 20's region and also possesses a 15-residue tail. The small size of *c*<sub>551</sub> is due to a massive deletion (39–58 in tuna cytochrome *c* numbering) of a loop at the bottom of the molecule. The folding of

the rest of the molecule is however conserved and *c*<sub>551</sub> is clearly related to the other cytochromes.

Dickerson *et al.* go on to propose that this same deletion probably occurs in cytochrome *f* (involved in algal photosynthesis), *c*<sub>555</sub> (a photosynthetic cytochrome of the green sulphur and purple sulphur bacteria), *c*<sub>553</sub> (from the sulphate respirer *Desulphovibrio*) and the *c*<sub>s</sub> of *Pseudomonas mendocina*. This assumption makes the sequence alignment of all these cytochromes with eukaryotic cytochrome *c* much more credible and suggests the existence of a large evolutionarily homologous group of cytochromes whose members all possess a similar polypeptide folding pattern (the cytochrome fold). It is tempting to assume that the different electron transport pathways in which these diverse cytochromes operate also have an evolutionary connection and Dickerson *et al.* explore the way in which their data can be interpreted in terms of the development of bacterial energy metabolism.

Dickerson *et al.* have drawn attention to the remarkable sequence work of Ambler and colleagues (Ambler *Handbook of Biophysics and Molecular Biology*, 1976; Ambler, Meyer and Kamen, *Proc. natn. Acad. Sci. U.S.A.*, in the press) on other cytochromes *c*<sub>2</sub> in the purple-non sulphur bacteria. Some of these *c*<sub>2</sub>'s align almost exactly with eukaryotic cytochrome *c* (*Rhodococcus vannelli*, for example) whilst other *c*<sub>2</sub>'s have, like *Paracoccus* *c*<sub>550</sub>, additional residues around residues 20, 50 and 70 (such as *Rhodospseudomonas capsulata*). In fact this range of structural variation within *c*<sub>2</sub> has now been extended by the finding of Ambler and Meyer (personal communication) that the *c*<sub>2</sub> of *Rhodospirillum tenue* has the massive deletion characteristic of *c*<sub>551</sub>. We are then left with the intriguing observation that the range of structural variation within cytochromes *c*<sub>2</sub> of the purple non-sulphur bacteria is as great as in all other cytochromes possessing the cytochrome fold.

Dickerson *et al.* consider that the main evolutionary implication of this homology is that bacterial and eukaryotic respiration arose from the dual function cyclic photophosphorylation and respiratory electron transport chain of purple non-sulphur bacteria by loss of the photosynthetic capability.

This interpretation confirms previous schemata for prokaryote evolution (such as Hall, *J. theor. Biol.*, **30**, 429–454, 1971 and in the *Evolution of Bioenergetic Processes*, edit. by Broda, E., 1975) but the evidence really hinges on the question of whether the different cytochromes *c* are evolutionarily representative of the whole electron transport pathways of which they are

members or whether this phylogeny has been muddled by genetic transfer. Physiological studies within the purple non-sulphur bacteria, which have representatives of each major type of cytochrome should resolve this question to some extent. Nevertheless, the purple non-sulphur bacteria could be the key to our understanding the molecular evolution of cytochrome *c*.

## Palaeomagnetic diversity

from Peter J. Smith

PALAEOMAGNETIC studies were begun early this century in an attempt to determine the characteristics of the Earth's magnetic field before the few hundred years of direct observation. By the 1960s, however, they had come to be associated more particularly with the growing evidence in favour of continental drift and seafloor spreading. With the now-widespread acceptance of the principal tenets of the new global tectonics, there has been a tendency for palaeomagnetism to revert more frequently to its original aims, although it is still used widely to elucidate possible continental and/or polar movements (especially those preceding the onset of Wegenerian drift) and has, in addition, become a useful tool for solving particular geological problems (for example, in stratigraphy).

This modern diversity of roles is well illustrated by the many reports that have appeared in the past few months alone. Roy *et al.* (*Geophys. Res. Lett.*, **2**, 537; 1975), for example, have carried out a straight palaeomagnetic directional study of the world's oldest red beds—argillites, 2.3 billion yr old, found in the Huron Supergroup of Ontario. Perhaps 'straight' is hardly the right word in this context, for it is seldom easy to obtain a reliable direction from rocks of such an age. In this case, however, a combination of thermal, chemical and alternating field cleaning appears to give a reliable ancient pole at 67°N, 158°E from a magnetisation acquired at, or within a few tens of millions of years of, the formation of the beds. The new pole is ~100 Myr older than the oldest previous Laurentian poles; and its significance is that in the context of previous poles it provides evidence that the apparent polar wandering path relative to Laurentia from 2.3 to 1.9 billion yr ago was mainly latitudinal and from north to south.

Paradoxically, not only can it be

difficult to obtain a good direction from very old sediments because of their age, it can be equally difficult to obtain a good direction from very young sediments because of their extreme youth. The problem is that the most recent sediments are still unconsolidated and thus subject to erosion, slumping and many other processes likely to distort whatever magnetisation they may already have acquired. Moreover, the 175 1-m cores taken from the top of the sediment in Lake Geneva by Creer *et al.* (*Earth planet. Sci. Lett.*, **28**, 127; 1975) were also disturbed during handling and kept in storage for more than a year before any palaeomagnetic measurements were made on them. It is thus all the more surprising that no less than 39 of these cores gave "satisfactory and interpretable" declination-depth curves. Comparison of these results with the historic-archaeomagnetic declination curve has enabled Creer and his colleagues to estimate the sedimentation rate and its variation in Lake Geneva over the past 400 yr.

By contrast, Thompson's (*Geophys. J.*, **43**, 847; 1975) measurements of magnetic declination in lake sediments have been concerned with a much longer time scale. Cores from Lake

Windermere, Blelham Tarn and Ennerdale Lake in NW England and from Lough Neagh in Northern Ireland show that an oscillation in declination (about true north) with a period of about 2,700 yr has been occurring for at least the past 10,000 yr. Such oscillations are probably due to a combination of dipole wobble, drifting non-dipole sources and stationary intensity-varying non-dipole centres—which presumably explains why declination-time curves from Britain, Switzerland, the Aegean Sea and the Black Sea are difficult to correlate. However, as long as the core magnetisation is stable and at least one horizon is dated absolutely or cross-correlated, recent NW European sediments may be dated against the British declination mastercurve.

Thompson's sediments were all normally magnetised, of course; it is necessary to go back a few more thousand years to the most recent (short) reversal. Not that the reversal pattern is yet perfectly clear, even for the past few million years. For example, the Olduvai normal event within the Matuyama reversed epoch was originally dated at  $\sim 1.9$  Myr and the subsequently discovered Gilsa event in the same epoch was dated at about 1.6 Myr. In short, there appeared to be two separate events—a view sup-

ported by an early report suggesting that the Gilsa type section in Iceland included two normal flows possibly separated by a reversed one.

Unfortunately, it has since become clear that the original Olduvai date may not be accurate; indeed, the wide limits now placed on it are such as to throw doubt on the whole existence of two separate events. This doubt has now been reinforced by Watkins *et al.* (*Earth planet. Sci. Lett.*, **27**, 436; 1975) who have re-examined the Gilsa section and find that although there are two normal flows there is no reversed flow between them. Thus only one normal event is definitely represented (at  $1.58 \pm 0.08$  Myr); and the question of whether the Gilsa and Olduvai events are identical or distinct remains open.

Returning now to the almost single (normal) polarity of the Brunhes epoch, Watkins and Richardson (*Geophys. J.*, **43**, 501; 1975) have been looking again at the contrast in palaeomagnetic data between the northern and southern hemispheres. Second-order differences between hemispheres were first discovered by Wilson (*Geophys. J.*, **19**, 417; 1970 and **22**, 491; 1971) who concluded that during the Upper Cainozoic the axial dipole was displaced along the rotational axis

ONE cannot help admiring the way certain species of plant and animal have exploited to their own advantage situations created by man. Even the most ugly and destructive activities have provided opportunities for those species with a sufficiently wide range of tolerance or the capacity to evolve such a tolerance. As a result urban environments have become populated by species such as the starling whose cliff and tree nesting and roosting habits, coupled with the capacity to feed in short turf, have provided it with a competitive opportunity. Among plants, the rose bay willow herb (*Chamaenerion angustifolium*) is a particularly successful invader of demolition sites, arriving as wind borne seeds and propagating vegetatively once established by means of stolons which give rise to erect stems. It is intolerant of shade and its natural habitat is open scree slopes. This is one of the many weed species which has survived in open localities since the close of the last glaciation and has spread extensively as man has provided it with new opportunities.

The mining of heavy metals and the tipping of spoil has created habitats which are toxic and inhospitable to most plant species, but some appear capable of evolving tolerance to such conditions and expand their populations as a result. For example, the bladder

campion (*Silene vulgaris*), another periglacial species turned weed, has developed races tolerant of high zinc concentrations and able to invade mine spoil (Gries, *Flora, Jena*, **156**, 271; 1966). A similar process has been observed operating in a number of species, particularly grasses, in the British Isles (Bradshaw *et al.* in *Ecology and the Industrial Society*, edit. by Goodman, G. T., Edwards, R. W., and Lambert, J. M., 327, Blackwell, Oxford; 1965).

Orchids are not normally regarded as a group of plants exhibiting heavy

## Orchids adapting

from Peter D. Moore

metal tolerance, but some recent observations by Richards and Swan (*Watsonia*, **11**, 1; 1976) in Northumberland in the north of England have altered this. They have found the narrow-lipped helleborine (*Epipactis leptochila*) growing on eleven sites, 180 miles north of the nearest previously recorded station. All the sites consisted of bare, open river gravels and most of them contained very high levels of zinc (up to 2,300 p.p.m. air-dry soil), and had a neutral pH.

The characteristic habitat of *Epi-*

*pactis leptochila* is beneath beech canopies on chalk and limestone in southern Britain. The plants in Northumberland could be derived from some previously undiscovered, relict population, or they could have invaded directly from the south of England. Richards and Swan consider it possible that the species has been in the area for 80 yr or more, having re-examined older, less critical identifications, but the origin of the species in the area remains uncertain. What is of greater importance and interest is the development of tolerance to zinc and its resultant success in polluted gravels where mining activities have occurred. At such sites the general vegetation is sparse due to toxicity, hence there is little competition from other herbaceous species, which is one character these northern sites have in common with the southern habitat of *Epipactis leptochila*.

As Bradshaw *et al.* have shown, not all plant species possess the ability to adapt to soils polluted with toxic metals, but evidently *Epipactis leptochila* does, and the Northumberland populations must have been subjected to considerable selective pressure for zinc tolerance. Like the starling and the rose bay willow herb, this is a species with latent talents that is now proving its worth by exploiting habitats made derelict and inhospitable by man. □

to the north by  $285 \pm 74$  km. Later, however, Watkins (*Geophys. J.*, **28**, 193; 1972) pointed out that, although the northern hemisphere palaeomagnetic results for the Brunhes alone are consistent with a dipole offset to the north by 300–400 km, the southern hemisphere data require no such offset. He therefore suggested that the simplest model consistent with data from both hemispheres would have a main centred dipole with a weaker axial dipole some distance to the north.

In a more thorough analysis, Watkins and Richardson now show that the Brunhes palaeomagnetic data are best satisfied by a major dipole offset to the north together with two minor axial dipoles offset arbitrarily to the core-mantle interface (one in each hemisphere). These minor dipoles have moments of 1–4% of that of the main dipole, the one in the southern hemisphere having the same polarity as the main dipole and the one in the northern hemisphere opposing the main dipole. This is apparently the simplest model consistent with all current Brunhes palaeomagnetic results although more complex models could be devised (and may be necessary later to explain better data).

Finally, Bhattacharyya and Leu (*J. Geophys. Res.*, **80**, 4461; 1975) have used the magnetism of rocks indirectly to investigate a much more local effect. From an analysis of magnetic anomalies over Yellowstone National Park, they show that the Curie point isotherm below this geothermal area is particularly shallow. This is only to be expected and in any case has been proved before using other methods. The point of this confirmation, however, is its suggestion that aeromagnetic data may prove useful in regional reconnaissance for potential geothermal energy resources □

## Tail-wagging antibodies?

from C. C. F. Blake

Now that X-ray studies have established a broad understanding of the antigen binding function of immunoglobulins, attention is being turned to the Fc region and how its complement fixation and B-cell activation functions are influenced by binding of antigen.

The first successful structural study of a whole myeloma protein, the IgG1 Dob, by both low resolution X-ray analysis and electron microscopy (Sarma *et al.*, *J. biol. Chem.*, **246**, 3753; 1971; Lebow and Davies, *J. ultrastr. Res.*, **40**, 349; 1972) revealed that the molecule was T-shaped, but little else. Recently Huber and his

colleagues (Colman *et al.*, *J. molec. Biol.*, **100**, 257; 1976) reported a second low resolution study, of the IgG1 Kol, which has produced much more information. There are two reasons for this. first, high-resolution structural information on the Fab region is now available and second, the Kol protein does not have the deletion in the Fab-Fc hinge region that the Dob protein has. Since the resolution of Huber's map is only 5 Å, only the quaternary structure of the molecule—the arrangement of the various domains—can be analysed in any detail. Changes at this level of structure, however, could be highly significant, and some very intriguing results have been obtained, even though, or rather because, there is no electron density for the Fc region of the molecule.

One of the characteristics of X-ray analysis of crystal structures is that it is only possible to see side-chains or parts of molecules that are positionally stable in the crystal. Thus the lack of electron density in that part of the crystal that should contain the Fc of Kol, must indicate that the whole region can, and does, take up at least two orientations relative to the well-defined Fab regions. Huber has interpreted this as indicating the presence of a hinge in the molecule at the point at which the electron density fades out—immediately C-terminal of the inter-heavy chain disulphide bridges. If this is so the hinge is different from the classical one proposed to account for the Y→T transformation which must be N-terminal of these bridges. It should be noted that the angle between the two Fab arms is  $125^\circ$  in Kol, in contrast to the  $180^\circ$  found in Dob.

Yet a third hinge region may be present in the molecule, coincident with the switch regions between the V and C domains of the Fab arms. Interpretation of the Fab region of the Kol protein in terms of the known structures of the human  $V_k$  dimer fragment Rei, and the mouse McPC 603 Fab fragment, has shown that the V and C domains are not in the same relative orientation as that found in the Fab fragments so far analysed. The difference can be judged by the angle of intersection of the pseudodyads that relate the light and heavy chains in the two domains, which changes from  $120^\circ$  in the Fab fragments to  $170^\circ$  in the Kol protein. Colman *et al.* refer to this change as “bending the elbow” of the Fab arms—in the Kol protein therefore the arms are almost straight.

Huber and his colleagues speculate that these two new hinge regions may permit antibodies to act as allosteric proteins, in which the allosteric effect is transmitted through the polypeptide chain from one domain to another.

They suggest the possibility that hapten binding is accompanied by a “bending of the elbow” and that the elbow movement is sensed by the hinge causing a change in the Fc region that by implication influences complement fixation. Although there is little other evidence that complement fixation is accompanied by conformational changes, the present hypothesis is certainly plausible in view of the extraordinary domain structure of the immunoglobulins, and we now await the results of hapten binding to the Kol protein with considerable interest. □

## Tunguska revisited

from David W Hughes

At about 7.17 a.m. local time on June 30, 1908 in the basin of the River Podkamennaya Tunguska, Central Siberia (latitude  $60^\circ 55'N$ , longitude  $101^\circ 57'E$ ) a gigantic explosion occurred. The ancient trees of the mighty Yenissi taiga were torn up by their roots and in places piled up in thick layers by the explosion wave, their trunks pointing radially away from the centre of the explosion. The devastation extended over an area of radius 30–40 km, the centre of the area having been ravaged by fire, searing being traceable for 18 km. A farmer 60 km away told how his shirt was almost burnt off his back, the explosion throwing him off the steps of his house and several feet across the ground. Eye witnesses up to 500 km away saw, in a cloudless sky, the flight and explosion of a blindingly bright, pale blue bolide, “which made even the light of the Sun appear dark”. This left in its wake a thick dust trail. The explosion took the form of a vertical column of fire and threw incandescent matter up to a height of 20 km. The sound of the explosion, like gun fire, reverberated thousands of kilometres away, seismographs registered an earthquake; the explosion air wave, recorded on microbarographs in many meteorological stations, went twice round the world. Magnetic disturbances similar to those subsequently recorded after atmospheric nuclear explosions were recorded at the Irkutsk Observatory.

After the explosion the nights were exceptionally bright over Western Asia and Europe, the enhanced night brightness slowly diminishing and disappearing after two months. In mid-July, two weeks after the explosion, the coefficient of transparency of the atmosphere was found to be noticeably depressed over California. This, it was suggested, was due to the loss of vast amounts of material from the incident body as it

travelled through the atmosphere. Observations indicated this loss to be several million tons, a hundred times more than the normal annual influx of meteoric matter.

Fortunately the fall region was very sparsely populated, the only inhabitants being scattered bands of nomadic Evenki reindeer herders. Due to the untimely occurrence of political upheavals in Russia the first cursory inspection of the impact site did not take place for 19 years when eventually Leonid A. Kulik headed an expedition organised by the Academy of Sciences of the USSR.

Many expeditions have since visited the area and many papers have been written discussing the phenomenon and putting forward ideas as to its cause. One of the latest papers appeared in a recent issue of *Physics of the Earth and Planetary Interiors* (11, 61; 1975). In it Ari Ben-Menahem of the Adolpho Bloch Geophysical Observatory, Rehovot, Israel re-analyses the old seismograms of the Tunguska event and deduces the parameters of the original explosion by comparing the old data with contemporary records of the seismic and acoustic effects of the air explosion that took place at Lop-Nor, Sinkiang on October 14, 1907 and the series of air explosions from nuclear tests at the USSR test site at Novaya Zemlya (74°N, 150°E).

The Tunguska sound waves were of acoustic modes  $S_0$  and  $S_1$  and travelled at group velocities between 280 and 310 m s<sup>-1</sup>. The seismic event was excited by the air explosion at the source, energy being radiated outward from the epicentre by a series of wave motions. The Rayleigh mode, a rolling motion of the Earth's surface, moved at about 2.7 to 3.5 km s<sup>-1</sup>. SH body waves, where the displacements are horizontal and perpendicular to the direction of propagation were recorded only at Irkutsk and the time of their arrival, coupled with arrival times of other waves at other observatories gives, according to Ben-Menahem, the explosion time to be 00 h 14 m 28 s UT. This time differs by about 30 s from previous estimates. The time delay between the arrival of the SH wave and the Rayleigh wave indicates that the main explosion occurred at a height of 8.5 km above the ground. The direction and extent of fallen trees around the epicentre suggest that this phenomenon was caused by the superposition of two waves—a spherical shock wave (due to the explosion, equivalent to a vertical point impulse of  $7 \times 10^{18}$  dyne s) and a conical ballistic wave (due to the moving incident object, equivalent to a horizontal point impulse of  $1.4 \times 10^{18}$  dyne s). The extent of the damage and the magnitude of the acoustic and seismic waves

indicate that the explosion had an energy of  $(5 \pm 1) \times 10^{23}$  ergs, equivalent to  $12.5 \pm 2.5$  megatons of TNT.

Ben-Menahem does not leap into the controversy as to the actual form of the incident object simply referring to it as a "UFO". Jackson and Ryan (*Nature*, 245, 88; 1973) thought it might have been a black hole with the mass of a large asteroid ( $10^{20}$ – $10^{22}$  g) and a negligible geometrical radius. This would have shot straight through the Earth but unfortunately for the theory (although fortunately for us) the exit point, latitude, 40–50°N longitude 30–40°W, in the mid-Atlantic was not marked by an equally severe shock and blast wave. Hunt, Palmer and Penny (*Phil. Trans. R. Soc. Lond.*, A252, 275; 1960) consider the possibility of an extraterrestrial almost-critical mass of fissionable material becoming tamped on entering the atmosphere. It is difficult, however, to conceive how a sufficient amount of, say, deuterium and tritium can be compressed and heated to several million degrees centigrade and maintained in that state sufficiently long for the self heating to carry the reaction to the explosive stage merely by entry into the Earth's atmosphere. Cowan, Athuri and Libby (*Nature*, 206, 861; 1965) suggest that an incident anti-matter body annihilated itself in the atmosphere, but no large anti-matter bodies have, as yet, been observed and it is hard to understand how it penetrated to such a depth in the atmosphere and why the explosion maximised at the end of the trajectory and not midway along it.

The most likely theory, that a small comet struck the Earth, was put forward by Whipple (*Q. Jl R. Meteorol Soc.*, 56, 287; 1930) and Astapovich (*Astr. J.*, 10, 465; 1933). The trajectory of the bolide indicated that the comet was travelling in the opposite direction to the Earth and that the head-on collision was at a velocity of 60 km s<sup>-1</sup>. The cometary nucleus, which, according to Whipple, is a large dirty snowball—a loose collection of dust and rock interspersed with water, methane and ammonia ices—exploded above the Earth's surface. If most of the energy came from the dissipation of kinetic energy and only a negligible portion from the chemically explosive reactions between the air and the radicals in the nucleus then the mass of the incident comet can be calculated to be approximately  $3 \times 10^{10}$  g. The fact that this comet nucleus has a diameter of about 40 m, more than an order of magnitude below the diameters estimated for visual comets, explains why it wasn't seen as it approached on its collision course to the Earth.

Further evidence favouring this hypo-

thesis is that the night sky luminescence after the fall was only observed over Siberia, Russia and Western Europe. Calculations of the comet orbit indicate that the dust and gas tail, which is directed away from the Sun, extended in a north-westerly direction from the point of impact. The dissipation of this tail in the atmosphere increased the night sky brightness by about 50–100 times. The discovery of many small (5–450  $\mu$ m diameter) magnetite and silicate globules in soil samples taken from the Tunguska site also supports the comet hypothesis. These were formed when molten 'rain drops' of dust solidified as they slowly drifted to Earth after the explosion of the retarded nucleus. Flux estimates indicate that a cometary nucleus of this size will hit the Earth about every 2,000 yr, the rarity of the event giving ample justification for visiting Tunguska yet again.

## Far-infrared balloon astronomy

from a Correspondent

THE far-infrared region of the electromagnetic spectrum—wavelengths between 20  $\mu$ m and 1 mm—is probably the last regime to be explored by the astronomer. The reason for this apparent lack of resolve is not that the wavelength range is uninteresting—far from it when we realise that the majority of the many clouds of dust scattered throughout our Galaxy radiate most of their energy at precisely these wavelengths. The problems are technological; in the far-infrared, the Earth's atmosphere is virtually opaque to external radiation because of severe attenuation by water vapour molecules. There are very poor transmission windows at wavelengths of 350  $\mu$ m, 450  $\mu$ m, and around 800  $\mu$ m, but these are accessible only from very high, dry sites such as Mauna Kea at 14,000 feet in Hawaii. Even then, transmission figures better than 30% are very rare. The obvious answer is to observe from above the absorbing layers in the atmosphere, and with dramatic improvements in balloon astronomy, many exciting discoveries are being made.

The University College London (UCL) group, led by R. E. Jennings, has pioneered balloon far-infrared astronomy and its latest flights have produced a catalogue of far-infrared sources in the Galactic plane. The 40-cm telescope, operating at an altitude of about 100,000 feet is directed to raster scan selected regions of the Milky Way, most of these having



been chosen because they are known from radio observations to contain complex H II regions.

These ionised gas domains surrounding a recently formed, high luminosity star are most intriguing astronomical objects. The unravelling of the detailed mechanisms of these star formation centres occupies a considerable fraction of astronomical effort and the problems are many and complex. The cores of these regions are thick with dust, often rendering optical identification of the ionising star or protostar extremely difficult, if not impossible. Near-infrared observations however do often reveal stars and protostars surrounded by very hot dust shells whose signals can be the free-free radio continuum emission of the H II region. These regions are also rich sites of molecular clouds, and emission maps of many and various molecules have been obtained at wavelengths of a few millimetres or centimetres for the most intense sources. To attempt to piece together the energetics of the emission nebulae, one must determine the total luminosities of the gas, dust and molecules and so derive the luminosity of the exciting star or stars, which must be the powerhouse of the whole region.

The key to understanding these regions is the presence of dust. The dust grains may be associated with the Stromgren sphere of the H II region and in this case are being heated by the direct flux of stellar ultraviolet radiation or by Lyman  $\alpha$  radiation of the hydrogen gas surrounding the star. Alternatively, the grains could be associated with the dense molecular clouds found in and around H II regions, the grain heating being caused by stellar or protostellar objects within these molecular clouds. In the former case, the dust emission, peaking at say 100  $\mu\text{m}$ , should be centred on the position of free-free continuum emission, whereas in the latter, the far-infrared emission should follow the contours of the molecular emission.

Using the UCL balloon system, operating in the wavelength range 40  $\mu\text{m}$  to 350  $\mu\text{m}$ , Furniss *et al.* (*Astrophys J.*, **202**, 400; 1975) have produced the most detailed far-infrared map of the complex region W3 currently available. Their map reveals that the far-infrared emission seems to follow the radio emission in general, although there does appear to be a long spur projecting westwards from the main source. The authors claim that recent radio free-free emission has also been reported to possess this spur feature and so the tie-in with the radio emission rather than the molecular cloud emission would seem to be more favoured for this source at least.

This, however, is only one source and there are many others; the Great

Nebula region of M42 in Orion, for instance, one of the most exciting and complex regions in the sky, shows many differences to W3. It is also found that as one observes at longer wavelengths ( $\sim 1\text{ mm}$ ) one is looking at the cooler dust and this component does appear to follow the molecular cloud emission rather than the H II continuum free-free emission. Once again the complexities of the regions are daunting and challenging. Theoretically one is not at all sure what size are the dust grains in the regions, or of what material; silicates or graphites are the usual choice. Laboratory spectral analysis of selected grain compositions is urgently required by the grain theoretician. Observationally one requires better high resolution, far-infrared and molecular maps and a near-infrared search for the dust-enshrouded protostars in the H II regions before the vastly complex physics and mechanics of these birthplaces of stars can be understood fully. In the far-infrared, one looks to the future for balloon and aircraft-based spectrometers and interferometers. The larger diameter balloon-based telescopes and the first non-military, infrared satellite, scheduled for launch in 1981, will open up a new era of observing in this wavelength range.  $\square$

## System for steroids

from Robert Shields

An understanding of the way in which steroid hormones regulate gene expression has been frustrated continuously by the lack of suitable model systems in which hormone action can be studied *in vitro*. Ideally what is required are hormonally responsive cell lines where mutant cells can be isolated and the mechanism of hormone action can then be dissected in the way that proved so successful in prokaryotes. Several steroid responsive cell lines are now available, including some potentially useful mutants (Sibley and Tomkins, *Cell* **2**, 213; 1974) but they all have a number of drawbacks. Now it looks as if the endocrinologists' prayer is about to be answered, for not only is an almost ideal system available, but combining as it does both endocrinology and tumour virology it should hit the double grant jackpot.

Recently it has been shown that physiological concentrations of glucocorticoids can stimulate the production of mouse mammary tumour virus (MMTV) in a number of cell lines established from mouse mammary adenocarcinomas (Parks *et al.*, *Science*, **184**, 158; 1974). This increase in virus production results from increases in virus specific RNA transcribed from the DNA provirus integrated in the host cell genome (Ringold *et al.*,

*Virology*, **65**, 135; 1975; Parks *et al.*, *J. biol. Chem.*, **250**, 3330; 1975). Induction of this viral RNA is mediated through the classical steroid-receptor mechanism since binding of various glucocorticoids to the receptor and transfer of the receptor-bound steroid to the cell nucleus are correlated with the increase in virus production (Young *et al.*, *J. biol. Chem.*, **250**, 3337; 1975; Ringold *et al.*, *Cell*, **6**, 299; 1975). Whether the increases in virus specific RNA produced by glucocorticoids involves a decrease in RNA degradation or an increase in transcription (which seems more likely) is not yet known. This is a vital point since transcriptional compared with post-transcriptional control has been a contentious point in steroid action for some time (see News and Views, *Nature*, **258**, 477; 1975). In any event the action of the steroid appears to be direct since inhibitors of protein and DNA synthesis do not block increases in viral RNA, no general increase in RNA synthesis is seen, and the concentration of the virus specific RNA doubles within 30 min of steroid addition. This effect of glucocorticoids is not confined to B-type RNA tumour viruses (such as MMTV). Glucocorticoids also increase C-type RNA virus produced in cells treated with halogenated pyrimidines (Paran *et al.*, *Proc. natn. Acad. Sci. U.S.A.* **70**, 2391; 1973) and enhance the production of polyoma (DNA) virus from productively infected mouse embryo cells (Morhenn *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1088; 1973).

What is particularly interesting about these systems is that steroids appear to be unable to initiate the transcription of virus sequences, and merely enhance the production of viral RNAs that are produced constitutively. Hence synthesis of C-type virus in KA31 non-producer cells is not induced by glucocorticoids but if virus is first induced with IUdr, virus production is increased many-fold by the hormone. Nor is constitutive production of viral RNA sufficient for a hormone effect since in murine epithelial cell lines constitutively transcribing both B-type and C-type virus sequences only the B-type transcripts are increased (Parks *loc cit*). Thus it may generally prove to be that steroids are only gene amplifiers and not true inducers.

Glucocorticoid control over virus production is a particularly good model system because viral RNA can be quantitated and tumour virologists have all the tools necessary to characterise mutant cells defective in various stages of viral production. In the not too distant future it may prove possible to remove the DNA provirus from the cell, together with its steroid responsive control elements.  $\square$

# articles

## X-ray studies of linear polyiodide chains in $\alpha$ -cyclodextrin channels and a model for the starch-iodine complex

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*The crystal structures of complexes of  $\alpha$ -cyclodextrin (cyclohexaamylose) with  $\text{Li}^+$ - and  $\text{Cd}^{2+}$ -polyiodide have been analysed by single crystal X-ray methods. The molecules are arranged head to head and linked by hydrogen bonds yielding endless stacks with channel cavities of about 5 Å diameter in which the iodine chains are located. In the  $\text{Li}^+$  complex, the polyiodide chain is slightly zigzagged.*

LINEAR polyiodine and polyiodide chains included in organic matrices are interesting for their unusual physical properties. The best known examples in analytical chemistry are the "blue starch-iodine" reaction, and in optics the polarisation filters based on complexes between polyiodide and organic polymers<sup>1</sup>. The nature of the blue or black colour of these compounds has been studied spectrophotometrically<sup>2</sup> and explained by the electron gas theory<sup>3</sup>. The one-dimensional metallic properties, such as anisotropic conductivity and weak paramagnetism<sup>4</sup>, of polyiodide and polyiodine<sup>5</sup> chains have been pointed out. As no detailed picture of an isolated linear polyiodide chain<sup>6</sup> had been developed, it seemed necessary to study the  $\alpha$ -cyclodextrin-polyiodine complex which preliminary studies have indicated contain well defined polyiodide chains<sup>3,7</sup>. (Reddy *et al.*<sup>8</sup> found linear chains of polymerised  $(\text{I}_3^-)_n$  units in the  $\text{HI}_3$  benzamide complex. These chains, however, occur in pairs which are stabilised by proton bridges.)

$\alpha$ -Cyclodextrin (cyclohexaamylose,  $\alpha$ -CD) is obtained through degradation of starch by *Bacillus macerans* amylase. It consists of six  $\alpha$  (1 $\rightarrow$ 4) linked glucoses (Fig. 1) and forms a truncated cone with height 8 Å, diameter 14 Å and an annular aperture of 5 Å. Owing to this aperture  $\alpha$ -CD can form inclusion complexes with small molecules<sup>3,7</sup> and these complexes crystallise in cage or channel structures, depending on whether the  $\alpha$ -CD molecules are arranged crosswise or stacked on top of each other like coins in a roll<sup>9</sup>. The channel type structures bear some relationship to the helical starch molecules for the  $\alpha$ -CD molecules form a hollow cylinder, with a geometry similar to that deduced for starch<sup>9</sup>.

When  $\alpha$ -CD is crystallised from aqueous solution in the presence of iodine, cage type crystal structures with isolated iodine molecules are formed<sup>10</sup>. When metal iodides are added to the solution, channel type structures with polyiodide chains develop, exhibiting either triclinic, tetragonal or hexagonal space group symmetries, depending specifically on the added cation (W. S., U. Bergmann, M. N. and P. C. Manor, unpublished). This article describes the crystal structures of the

complex  $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$  (triclinic) and  $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 26\text{H}_2\text{O}$  (tetragonal).

### Polyiodide chain in $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$

Table 1 shows data for the triclinic, pseudohexagonal crystals of  $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot \text{I}_2 \cdot 8\text{H}_2\text{O}$ . The heavy atom structure has been solved using tangent formula refinement of the rough model obtained from a Patterson distribution while the  $\alpha$ -CD atoms, the water molecules and the cation had to be located from difference Fourier syntheses. The structure was refined by full matrix least squares cycles to a discrepancy index  $R = 8.8\%$ .

The crystal structure of the complex is determined by hexagonally packed  $\alpha$ -CD stacks parallel to the crystallographic  $c$  axis with unit cell repeat distance 15.88 Å (Fig. 2a). In these stacks, the  $\alpha$ -CD molecules are tilted by about 7° against the

Fig. 1 Chemical structure and nomenclature of  $\alpha$ -CD.

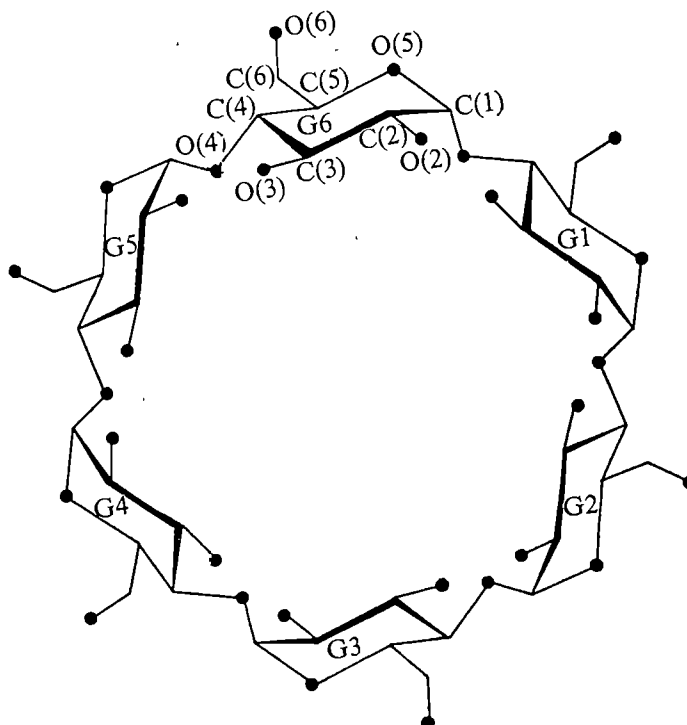


Table 1 Crystallographic data

Complex	( $\alpha$ -CD) $_2$ ·LiI $_3$ ·8H $_2$ O	( $\alpha$ -CD) $_2$ Cd $_{1/2}$ ·I $_2$ ·26H $_2$ O
Habitus	Hexagonal plates or needles	Needles with rectangular cross section
Colour	Black-brownish, metallic lustre	Black-greenish, metallic lustre
Space group	Strong dichroism P1	Strong dichroism P4 $_2$ 2 $_1$ 2
Cell constants	a = 13.88 Å* b = 13.88 Å c = 15.69 Å $\alpha$ = 94.1° $\beta$ = 87.8° $\gamma$ = 119.9°	a = b = 19.93 Å* c = 30.87 Å
Density calculated	1.72 g cm $^{-3}$	1.69 g cm $^{-3}$
Density measured	1.72 g cm $^{-3}$	1.70 g cm $^{-3}$
X-ray data collected	5,600	3,400
Final R factor	8.8%	12.0%

\*a (tetragonal)  $\approx \sqrt{2}$ ·a (triclinic); c (tetragonal)  $\approx 2$ ·c (triclinic)

stack axis, thereby prohibiting a truly hexagonal space group. The  $\alpha$ -CD molecules are arranged head to head to form dimers which include five iodine atoms (Fig. 3) and are linked along the stacks through intermolecular O(2)  $\cdots$  O(3) and O(6)  $\cdots$  O(6) hydrogen bonds and through bonds to the eight water molecules located in the 'empty' space between the stacks. Further, the Li $^+$  cations are coordinated to O(2) and O(3) hydroxyl groups of  $\alpha$ -CD molecules joined in dimers and to one water molecule, the ligands forming a distorted tetragonal pyramid; the shortest Li $^+$   $\cdots$  I distance, 5.05 Å exceeds ion-ion contact of 2.88 Å.

The individual  $\alpha$ -CD molecules display almost hexagonal symmetry. The glucoses are in the C1 chair conformation with all C(6)–O(6) bonds in the preferred *gauche*, *gauche* position<sup>11</sup>, that is they are pointing away from the  $\alpha$ -CD molecular centre (Fig. 3). The wide sides of the  $\alpha$ -CD cones are lined by the O(2) and O(3) hydroxyl groups which form a ring of intramolecular hydrogen bonds between adjacent glucoses with O  $\cdots$  O distances in the range 2.78–3.03 Å.

The arrangement of the iodine atoms in the polyiodide chain follows the inclination of the  $\alpha$ -CD molecules towards the stack axis (Fig. 3). In an  $\alpha$ -CD dimer, the positions of four iodine atoms lying in the narrow  $\alpha$ -CD cavities are fully occupied but the iodine atom near the wide O(2), O(3) rim of the  $\alpha$ -CD torus is twofold disordered at 70%: 30% occupancies. Since the two disordered iodine sites are only 1.7 Å away from each other they cannot be occupied simultaneously and at all events clearly belong to one of the neighbouring I $_2$  units; therefore, alternating I $_2$  and I $_3^-$  units can be distinguished.

As often observed, the I $_3^-$  units are not strictly linear<sup>12</sup> but bent to about 170° and the two I–I distances are not equal to each other. In the I $_2$  units, the standard I–I distance of 2.67 Å is lengthened to 2.81 and 2.87 Å and the van der Waals' separation between iodine atoms of 4.3 Å is shortened to at least 4.03 Å (Fig. 3).

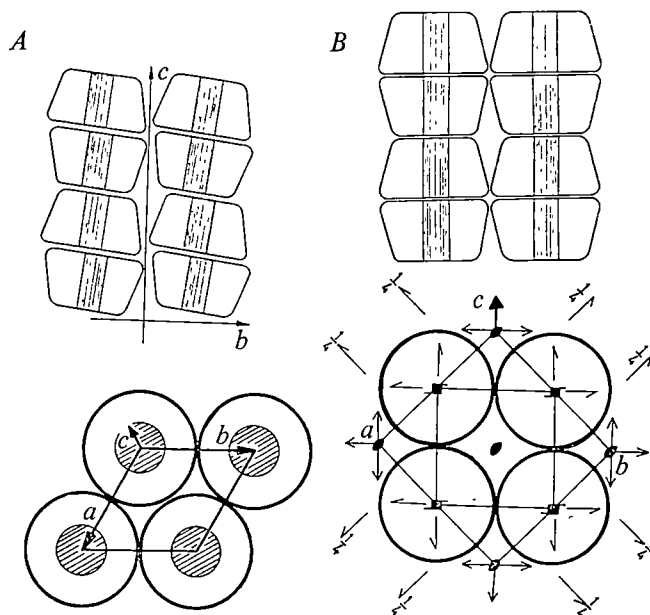
### Polyiodide chain in ( $\alpha$ -CD) $_2$ ·Cd $_{1/2}$ ·I $_2$ ·26H $_2$ O

The ( $\alpha$ -CD) $_2$ ·Cd $_{1/2}$ ·I $_2$ ·26H $_2$ O complex crystallises in the high symmetry space group P4 $_2$ 2 $_1$ 2. The heavy atoms were located by direct methods but the  $\alpha$ -CD molecule could only be found by trial and error. The cation and water molecules were located from difference Fourier syntheses and the atomic parameters were refined to  $R$  = 12.0%. Two half, independent  $\alpha$ -CD molecules constitute the asymmetric unit and are related to the other two halves by the diad component in the 4 $_2$  operation (Figs 2b and 4). Thus the  $\alpha$ -CD molecular axes coincide with the 4 $_2$  axis, the two  $\alpha$ -CD molecules again being arranged as dimers in a head to head fashion, but the intermolecular O(2)  $\cdots$  O(3) and O(6)  $\cdots$  O(6) hydrogen bonding pattern is not the same because the  $\alpha$ -CD molecules are rotated differently against each other (Figs 3 and 4). The dimers form stacks which are only loosely packed owing to the

requirements of the tetragonal lattice (Fig. 2b) and the open space between the stacks is filled by 26 water molecules per  $\alpha$ -CD dimer, some of them being statistically disordered. The Cd $^{2+}$  cation itself is twofold disordered, with both sites located on the twofold axes. They are coordinated octahedrally only by water molecules and > 9.97 Å from the polyiodide chain. The conformation of the  $\alpha$ -CD molecules themselves is similar, as discussed above.

Four of the five iodine atoms in the dimer are located on the 4 $_2$  axis and therefore they must be colinear. Similarly as in the ( $\alpha$ -CD) $_2$ ·LiI $_3$ ·8H $_2$ O complex, the iodine atom near the wide O(2), O(3) rim of the  $\alpha$ -CD torus is twofold disordered in equal amounts, which leads again to angles near 170° in the polyiodide chain. The two sites are too close together (1.24 Å) to allow simultaneous occupation (Fig. 4). In contrast to the separation into I $_3^-$  and I $_2$  units in the Li $^+$ -complex, however, the distribution of the iodine atoms follows a pattern, (I $_2$   $\cdots$  I $^-$   $\cdots$  I $_2$ ) $_n$ , usually considered as I $_5^-$ , with the iodide ion I $^-$  being disordered. The distances between the central iodine ion and the next iodine molecules, 3.17 and 3.14 Å, are comparable with previously observed I $_5^-$  structures<sup>13</sup>. The separation of 3.32 Å between the I $_5^-$  units is considerably shorter than the expected van der Waals' distance of 4.3 Å. These

Fig. 2 The packing scheme of  $\alpha$ -CD stacks in (A) the triclinic ( $\alpha$ -CD) $_2$ ·LiI $_3$ ·8H $_2$ O complex, (B) the tetragonal ( $\alpha$ -CD) $_2$ ·Cd $_{1/2}$ ·I $_2$ ·26H $_2$ O complex. The unit cell constants (a) and (b) are determined by the dimensions of the closely packed  $\alpha$ -CD molecules (see also Table 1).



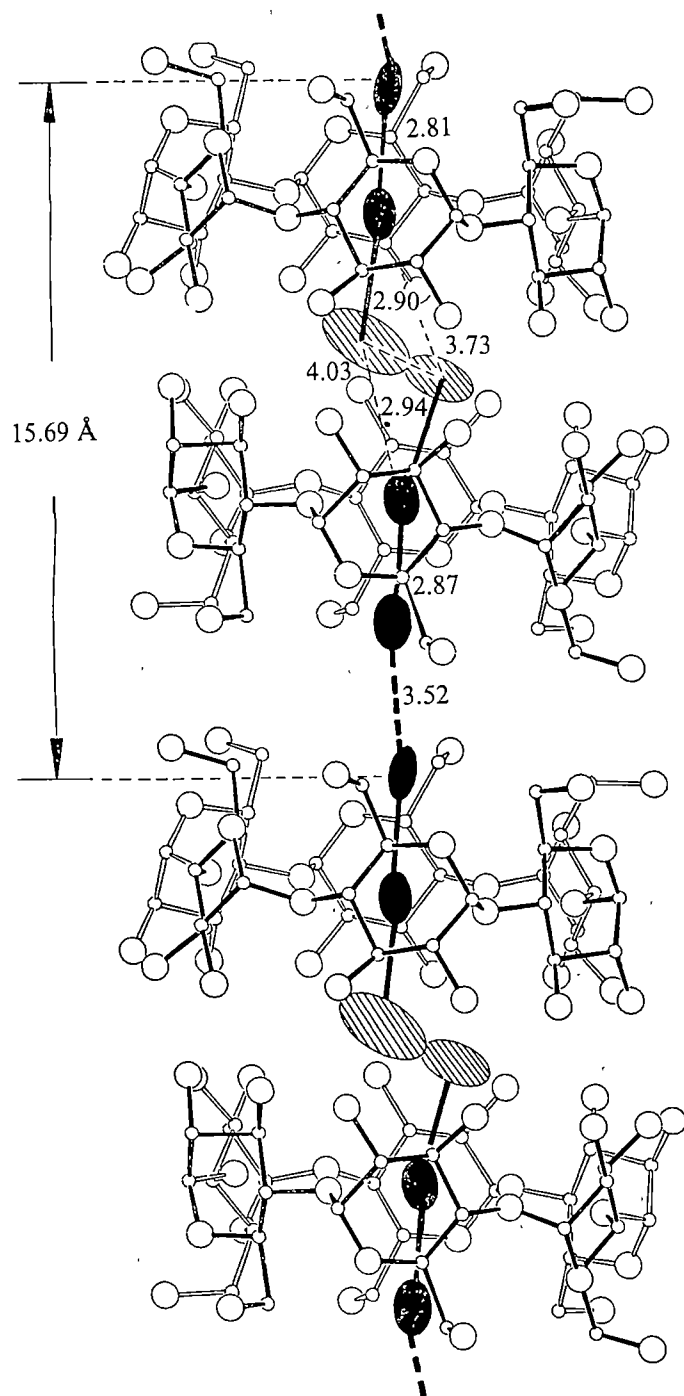


Fig. 3 The structure of the  $(\alpha\text{-CD})_2 \cdot \text{LiI}_3 \cdot 8\text{H}_2\text{O}$  complex, projected on the  $a,c$  plane, water molecules and  $\text{Li}^+$  ion not drawn. o, C; O, O; fully and statistically occupied iodine sites are indicated by filled and hatched ellipses representing the anisotropic thermal vibration of these atoms. Distances between iodine atoms are given in Å units. The crystallographic asymmetric unit is marked by dashed lines.

results indicate that in this polyiodide chain extensive delocalisation of electrons occurs.

### Conductivity measurements

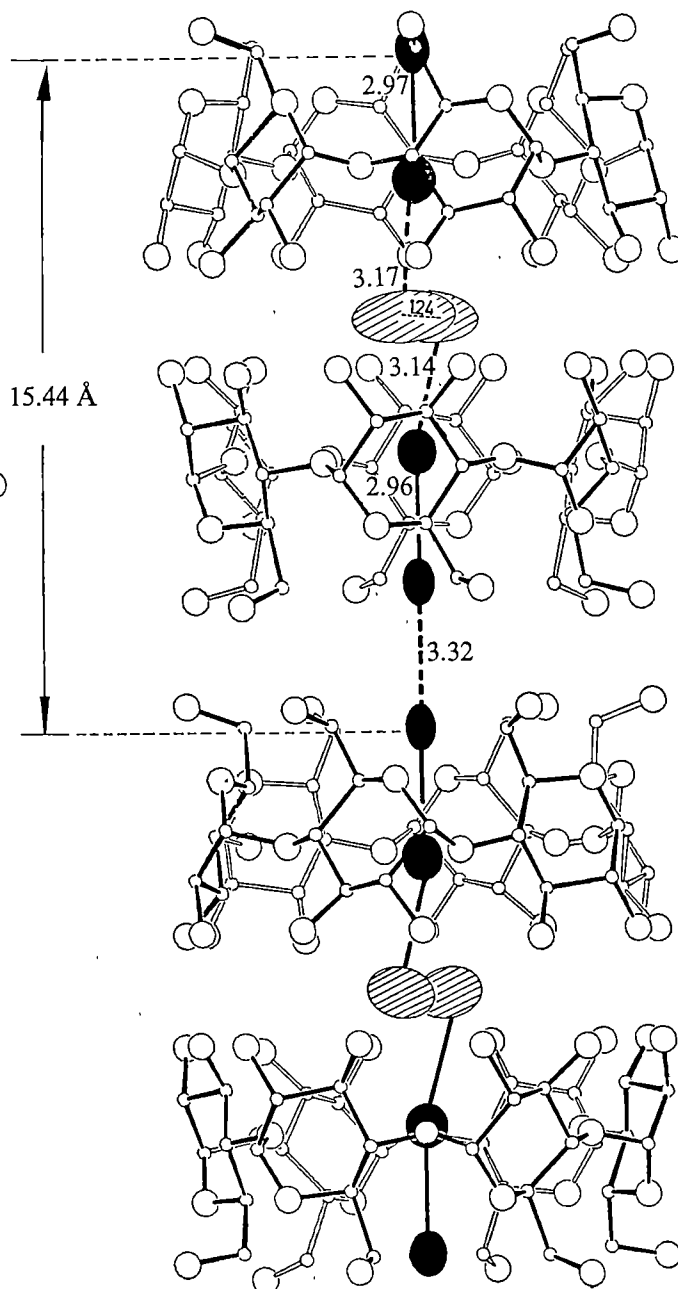
Several complexes of polyiodide with organic molecules have been investigated by X-ray crystallography<sup>13-15</sup>. In these structures the polyiodide chains consist of clearly distinguishable  $\text{I}_2$  and  $\text{I}_6^-$  units in a zigzag arrangement. Linear, isolated polyiodide chains<sup>6</sup> have been observed in detail only in the two crystal structures described above and in the trimesic acid

polyiodide<sup>15</sup> complex. Although in the two  $\alpha\text{-CD}$  complexes the iodine and  $\alpha\text{-CD}$  atoms were located and refined on the basis of three-dimensional X-ray data, the structure of the polyiodide chain in the trimesic acid complex was derived from one dimensional diffuse layer lines.

In the trimesic acid polyiodide complex, the periodicity along the polyiodide chain is also about 15.5 Å, five iodine atoms are arranged as centrosymmetric ( $\text{I}_2 \cdots \text{I}^- \cdots \text{I}_2$ ) units with I-I distances of 3.26 Å, 2.74 Å and 3.50 Å<sup>15</sup>, in close agreement with the distances observed for the tetragonal complex described above: 3.14 (3.17), 2.96 (2.97) and 3.32 Å. In the latter the tendency to achieve equidistant spacings is more obvious, and extensive delocalisation of electrons should be expected.

For that reason, preliminary conductivity measurements were carried out in the direction of the polyiodide chain of the  $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$  complex. The conductivity was about  $10^{-6} \Omega^{-1} \text{cm}^{-1}$ , in agreement with results obtained previously with the same complex (M. M. Labes, personal

Fig. 4 The structure of the  $(\alpha\text{-CD})_2 \cdot \text{Cd}_{1/2} \cdot \text{I}_2 \cdot 2\text{I}_2 \cdot 26\text{H}_2\text{O}$  complex. For further details, see legend to Fig. 3.





communication), and suggests that the polyiodide chain has the properties of a semiconductor. In summary, in strictly linear polyiodide chains  $I_6^-$  units with a fine structure  $(I_2 \cdots I \cdots I_2)^-$  seem to be preferred but in nonlinear, slightly zigzagged polyiodide chains  $I_2$  and  $I_3^-$  units can be distinguished. In complexes between polyiodide and polymer organic molecules such as starch or the polarisation filter polymers there are no crystallographic restrictions and it is to be assumed that slightly zigzagged chains will occur. It is an outstanding feature of the  $\alpha$ -CD-polyiodine complex that the cations are located outside the  $\alpha$ -CD channels at distances well beyond the expected  $Li^+ \cdots I^-$  or  $Cd^{2+} \cdots I^-$  ion pair interaction, yielding a negatively charged polyiodide embedded in the insulating organic material  $\alpha$ -CD. In other structures, the tendency to form ion pairs with polyiodide is obvious<sup>6,14</sup>.

## Intermolecular forces

Charge transfer interactions between iodine atoms and the ether-like O(4) linkages of  $\alpha$ -CD molecules have been reported to stabilise the  $\alpha$ -CD (or starch)-polyiodide complexes<sup>18</sup>. Our own and earlier<sup>10</sup> X-ray crystal studies show that the main interactions between iodine atoms and  $\alpha$ -CD molecules are van der Waals' contacts with the C(3)-H and C(5)-H methin hydrogen atoms: viewed from the iodine atom positions, the O(4) atoms are buried behind these hydrogen atoms and at least 0.68 Å further away than the  $I \cdots O$  van der Waals'

contact of 3.55 Å. Therefore, distances in the range between covalent and van der Waals' which are demanded for charge transfer type interactions<sup>17</sup> are not possible. As for the  $\alpha$ -CD-polyiodide complexes, it is unlikely that the starch-polyiodide complexes are stabilised by  $I \cdots O$  charge transfer interactions.

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# The role of long range forces in ordered arrays of tobacco mosaic virus

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*We examine the role of electrostatic repulsion and van der Waals' attraction in determining interviral spacings in 'equilibrium' gels of tobacco mosaic virus. The observed spacings are inconsistent with a force-balance model. Experimental probes of the role of forces are suggested.*

EVER since Bernal and Fankuchen's description, in 1941, of the parallel packing of tobacco mosaic virus (TMV) particles in the ordered phase of a two-phase system<sup>1</sup>, the TMV system has been considered an ideal example of spontaneous self-organisation. Since some biological structures, such as muscle sarcomere<sup>2,3</sup>, visual cornea<sup>4-8</sup>, and the gel of sickle cell haemoglobin (ref. 9 and A. P. Minton, personal communication), appear to be ordered arrays of long proteins or protein aggregates, one is tempted to make analogies between those structures and the array of the rod-like TMV particles. Possibly the observed spacings in TMV gels result from balancing the electrodynamic van der Waals' attraction, and electrostatic repulsion<sup>10-12</sup>. Primarily because of limitations in previous physical theory, this assumption has never been adequately examined. Here we have used new methods of computation to explore the role of long range forces in the TMV system.

There has been a fairly clear but arbitrary dichotomy in theoretical analyses. First, it has been shown that without any attractive force between rod-like particles, steric interference between asymmetric bodies is sufficient to cause a separation of the rod population into an ordered phase of parallel rods in

equilibrium with another phase of randomly oriented rods in suspension<sup>13,14</sup>. Second, work on long-range interactions has shown that energies of attraction can be comparable to the energy of Brownian (random) motion when bodies are close compared with their size<sup>15</sup>. Such interactions might therefore have a significant role in overcoming Brownian motion to align like particles.

The relative importance of these factors in the formation of the observed ordered arrays has remained an open question. The truncated virial expansion technique<sup>13,14</sup> used to describe the consequences of steric repulsion is not very accurate at the high particle concentrations at which observations were made. Such a treatment predicts transition densities, but cannot provide unambiguous values for the rod-rod spacing. The alternative lattice models are also not designed to predict observed spacings (ref. 16, and A. P. Minton, personal communication). Although experimental examination of uncharged polypeptides in organic solvents<sup>17</sup> shows that ordering of very long rods arises primarily from steric repulsion, none of the available hard rod models is able to account for the observed transition densities in TMV suspensions<sup>18</sup>.

In the following, we compare observed interviral separations with those predicted by force-balance. We use the theory of balancing forces to derive a constraint between separations and the salt concentrations in the medium, and we use recently published absorption spectra to make a numerical calculation of the van der Waals' attraction between proteins in salt water.

Two conclusions are immediately apparent: first, the magnitudes of the observed spacings are always much greater than

those expected from the positions of the energy minima. Second, the observed change in separation on varying the salt concentration in the medium cannot be fitted by shifts in the positions of equilibrium. (This is true even if one chooses unphysical coefficients for the competing forces.)

We suggest experiments for more critical examination of the role of forces, including repetition of the early spacing measurements<sup>1</sup> in more stringently defined conditions of pH and salt concentration in the medium.

### Force-balance constraint between spacing and salt concentration

The leading term in the electrostatic repulsive force per unit length between parallel rods is<sup>19</sup>

$$f^{es} = \frac{2v^2e^2}{\epsilon a} \left(\frac{2}{\pi}\right)^{1/2} \frac{\exp[-\kappa(R-2a)]}{\kappa R} \quad (1)$$

Equation (1) is valid when the rod length is much greater than the separation of the rods.  $R$  is the interaxial distance,  $a$  the rod radius,  $v$  the net number of unit ionic charges per unit length of the rod, assumed to be smeared uniformly on the outermost surfaces. The Debye constant  $\kappa$  is

$$\kappa^2 = \frac{8\pi ne^2}{\epsilon kT}, \quad n = \frac{1}{2} \sum_{\{n_i^0\}} n_i^0 z_i^2 \quad (2)$$

with  $(-e)$  the electronic charge,  $\epsilon$  the dielectric constant of the medium, which is assumed uniform,  $k$  the Boltzmann constant,  $T$  the absolute temperature, and the  $n_i^0$  are the concentrations of ions of valence  $z_i$  in the bathing medium far from the rods. Equation (1) assumes that the radius  $a$  is greater than the Debye length  $\kappa^{-1}$ , and that the electrostatic energy of a counterion near the rod is smaller than the thermal energy  $kT$ . Expressions more general than equation (1) have been derived and may be applied if needed<sup>10,19</sup>.

The electrodynamic attractive force per unit length between the same two rods has the approximate form<sup>10,20</sup>

$$f^{ed} = -\frac{2\pi A_H}{3R^2} S \quad (3)$$

where  $S$  is the double sum

$$S = \sum_{m=1}^{\infty} \sum_{n=1}^{\infty} \frac{[(2m+2n+1)!!]^2 (2m+2n-1)}{m!n!(m-1)!(n-1)!} \left(\frac{a}{2R}\right)^{2m+2n} \quad (4)$$

(The double factorial is the product  $q!! = q(q-2)(q-4) \dots (1 \text{ or } 2)$ .) Equation (3) neglects the retardation damping of van der Waals' forces. It also assumes that the dependence of the force on distance is obtained by pairwise summation of inverse sixth power interactions between incremental parts of the rods,

and that the rods are of uniform composition (although the formula may be very simply generalised to describe rods composed of concentric cylindrical layers, as might be appropriate for TMV particles). The magnitude of the coefficient  $A_H$  can be obtained from electromagnetic spectra of protein and water and will be computed below. 'Many-body' effects which might give a different form to  $f^{ed}$  will be negligible because of ionic screening of the low-frequency charge fluctuation forces most likely to demonstrate these effects. Again, more general formulae exist<sup>20</sup> and may be used if necessary.

If the interaxial spacings  $R$  observed by X-ray diffraction are the result solely of balancing electrostatic repulsion  $f^{es}$  and electrodynamic attraction  $f^{ed}$ , then these forces must sum to zero at the observed spacings  $R$ . Setting  $f^{es} = -f^{ed}$  and sequestering coefficients, we have

$$F(R, \kappa) = \frac{S/R^2}{\exp[-\kappa(R-2a)]/\sqrt{(\kappa R)}} \quad (5)$$

$$= \frac{v^2e^2}{A_H} \left(\frac{2}{\pi}\right)^{1/2} \frac{3}{\pi \epsilon a}$$

That is,  $F(R, \kappa)$  is a constant for all predicted pairs of values of  $R$  and  $\kappa$ . The equation

$$F(R, \kappa) = \text{constant} = C$$

provides a constraint between spacing and salt concentration depending on the value of the constant  $C$  where

$$C = \left(\frac{v^2}{A_H}\right) \left(\frac{2}{\pi}\right)^{1/2} \left(\frac{3e^2}{\pi \epsilon a}\right) \quad (6)$$

### Computation

We can compare the behaviour predicted in equation (5) with the available data on the TMV 'equilibrium gels'. Bernal and Fankuchen<sup>1</sup> provide a table of interaxis spacings as functions of salt concentrations; their results are presented in columns 1 and 2 of Table 1. The Debye constant corresponding to the various salt concentrations is given in column 3. The function  $F(R, \kappa)$  defined in equation (5) is then tabulated in the fourth column. According to the arguments given in the last section,  $F(R, \kappa)$  should be a constant if the spacing  $R$  corresponds to an energy minimum. This is not the case.

The criterion  $F(R, \kappa) = C$  is a very sensitive test of the force-balance picture. This can be seen in Fig. 1, where the values of  $R$  and  $\kappa$  satisfying  $F(R, \kappa) = C$  are plotted for  $C = 0.01, 0.1, 5, 50$ , and  $100 \text{ \AA}^{-2}$ ; the experimental points are included as well. Note that there are very few experimental points, and that a relatively small error in spacing suggests a large variation in  $C$ . In fact, any value of  $C$  between 5 and 100 seems to be a reasonable choice based on available data. Unreported experimental error might be one cause of the irregular behaviour of the points plotted on Fig. 1.

The points at 186 and 182  $\text{\AA}$  correspond to surface-surface distances that are much too small for the present theory to be quantitative. We have chosen a rod radius of 90  $\text{\AA}$  for the calculations. The TMV particle is a composite structure built of subunits some 25  $\text{\AA}$  apart at the viral surface<sup>21</sup>. These subunits protrude from the viral rod, and units from adjacent parallel rods can dovetail by 30  $\text{\AA}$  (the closest axis-axis distance is 150  $\text{\AA}$  for dry preparations, while accurate X-ray studies<sup>21</sup> on the wet particle suggest a particle diameter of 180  $\text{\AA}$ ). The minimum interaxial distance of 173  $\text{\AA}$  observed by Bernal and Fankuchen is probably accurate, but arises from a 7  $\text{\AA}$  dovetailing of the viral subunits. Given the graininess of the viral

Table 1

$\bar{R}^*$ ( $\text{\AA}$ )	Normality* equivalents ( $l^{-1}$ )	$\kappa$ ( $\text{\AA}^{-1}$ )	$F(R, \kappa)^\dagger$ ( $\text{\AA}^{-2}$ )
251-302	0.26	0.2054	$58.2-38.9 \times 10^4$
229	0.33	0.2313	6.9
223	0.52	0.2904	35.4
210	1.03	0.4077	86.3
186	2.06	0.5780	0.583
182	4.12	0.8174	0.324

\* Experimental values for interaxis spacing  $R$  and normality from Table III of ref. 1, salt =  $(\text{NH}_4)_2\text{SO}_4$ .

† See equation (5)

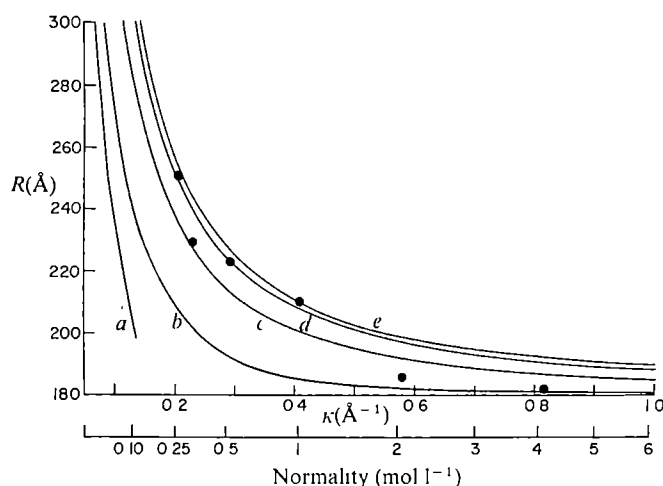


Fig. 1 The position of force-balance as a function of salt concentration in the bathing medium is plotted here for five values of the constant  $F(R, \kappa) = C$ . a,  $0.01 \text{ Å}^{-2}$ ; b,  $0.1$ ; c,  $5$ ; d,  $50$ ; e,  $100$ , ●, experiment. The experimental points of Bernal and Fankuchen<sup>4</sup> are included for illustrative purposes only, and not as evidence for the propriety of the force-balance picture. The curve for  $C = 0.01 \text{ Å}^{-2}$  ends abruptly at  $R = 198 \text{ Å}$  because no energy minima are found at smaller separations (attraction is always greater than repulsion).

surface it is inappropriate to use equation (5), which assumes a smooth cylindrical surface, for separations  $< 200 \text{ Å}$ .

The theory above assumes that the viral rods will interact in pairs, which is in accord with Coulombic repulsions in salt solutions where the density of the mobile ions exceed the volume density of charges fixed to large particles, that is, where the Debye length of the medium is less than distances between the large bodies. (In fact the salt concentrations used experimentally are probably too high for equation (1) to be used with confidence for the repulsion of two viral particles.) The assumption of pairwise rod-rod attraction conforms well with the criterion that the polarisability of the whole gel is linear in rod density<sup>22</sup>.

### Attractive forces and energy minima

Is there any *a priori* estimate of the constant  $C$  using equation (5)? For computation of the repulsive force, we know that there are probably between one and two ionic charges per protein subunit (D. L. D. Caspar, personal communication). We have estimated a Hamaker coefficient in the context of the Lifshitz formalism<sup>23-27</sup> using spectroscopic data for water at frequencies at up to  $25 \text{ eV}$  (refs 28-29), and for bovine serum albumin in the frequency range  $2-82 \text{ eV}$  (ref. 30). These data were fitted to the form of damped linear oscillators by the method of Knott and Shrager<sup>31,32</sup>.  $A_H$  was then computed by numerical integration, using the theory of Dzyaloshinskii *et al.*<sup>23-27</sup>. We neglected retardation damping of the van der Waals' force and assumed complete screening<sup>33-36</sup> of the low frequency fluctuations by salt.  $A_H$  was found to be between  $5 \times 10^{-14} \text{ erg}$  and  $9 \times 10^{-14} \text{ erg}$  depending on the accuracy of the optical data.

For a rod with 2,130 subunits per  $3,000 \text{ Å}$  length and a  $90 \text{ Å}$  radius<sup>21</sup>, the constant  $C$  from equation (7) will be roughly between  $0.01$  and  $0.1 \text{ Å}^{-2}$ . This range of values is lower than all values in Table 1 whatever the spacing and salt concentration. That is, inter-rod distances are always greater than those expected from force-balance. Note that  $C$  is independent of the length. This is helpful since viral length can be indeterminate because of end-to-end aggregation of viral particles<sup>37</sup>. Since our theory does not apply to the experimental conditions of Bernal and Fankuchen, one cannot draw any firm conclusions. The erratic estimates of the 'constant'  $C$  in Table 1 fall, however, well outside the reasonably expected range.

It further seems that well depths (the interaction energy at the equilibrium separation) are not large, but only  $\lesssim kT$  (ref. 10).

This may be compared to the guess of  $50 kT$  proposed in early work on force balance in muscle<sup>11</sup>. Inclusion of retardation screening of van der Waals' attraction would only weaken the depth of the expected energy minimum.

### Discussion

We expect that attractive forces will not strongly influence the arrangement of rod-like proteins at interaxial spacings  $> 200 \text{ Å}$ . They may be important in circumstances of high concentrations of weakly charged particles. It is now possible to delineate those circumstances more precisely, and to develop a better intuition about the organisation of proteins in experimental systems less accessible but more functional than TMV suspensions.

The most critical examination so far on long range forces in TMV gels is the 1953 study of Gerald Oster<sup>37,38</sup>. Working in a regime of low virus concentrations (2.5% by weight), and very low salt concentrations ( $\sim 5 \text{ mM}$ ), he observed that the addition of electrolyte destroys the gel even while screening repulsive forces. He concluded that repulsion is the dominant factor in ordering; that to within experimental accuracy the Onsager model gave accurate estimates of viral concentrations in both phases, and that attractive forces need not be considered until fairly high viral concentrations, where, in addition, there was almost no electric charge on the particles. In this regime a third, precipitated, iridescent phase has also been observed in TMV, the existence of which is sensitive to electrolyte in the medium<sup>38</sup>.

Our theory supports Oster's view and predicts negligible attraction between cylindrical particles in dilute solution. Since the interesting cases for the packing of linear proteins in other systems occur at higher volume fractions and higher salt concentrations than in Oster's work, it would be worthwhile to extend his observations to this regime to check our theoretical argument.

On this basis, it is possible to describe the experimental situations which would be most amenable to direct analysis. Specifically, one should work with low salt concentrations (probably  $< 0.5 \text{ M}$ ), low net charge densities on the rods (near the isoelectric point,  $\text{pH } 3.3$  (ref. 1, noting that there is zero net charge at  $\text{pH } 4.5$  when salt is absent from the bathing medium (D. L. D. Caspar, personal communication)) at interviral distances larger than the distance between viral subunits ( $21 \text{ Å}$ , ref. 21), and using solutions of monovalent salts rather than divalent or mixed valence salts. In these conditions, one may measure mean interaxial distances  $R$  for rods in the ordered (gel) phase of a two-phase system. These should be measured as a function of salt concentration (or Debye constant  $\kappa$ ) at all  $\text{pH}$  where a two-phase system exists.

The determination of phase diagrams for TMV in water at various  $\text{pH}$  and salt concentrations can facilitate theoretical study. The concentrations of protein in regions of two phases (ordered and disordered) need to be mapped as a function of  $\text{pH}$ , salt concentration and temperature.

The concentration of weakly charged viral particles in the dilute disordered phase can indicate the depth of any significant interparticle energy. The repulsion-only theory suggests that there will be of the order of one particle per  $\pi l^2 d/4$  volume where  $d$  and  $l$  are the effective particle diameter and length. Note that the diameter  $d$  is here an effective diameter, because of electrostatic repulsion, and  $l$  may be an effective length due to end-to-end aggregation<sup>37,39</sup>. They will be greater than the physical dimensions of the virus and be sensitive to salt concentration. If there is a strong binding (a negative energy  $-W$ ) between each particle with its neighbours in the ordered phase, the concentration in the dilute phase will go down as  $\exp(-W/kT)$  and  $W$  will be sensitive to salt concentration. Since this argument is only approximate, the existence of  $W$  will not be demonstrated unless the dilute concentration is qualitatively less than predicted for  $W = 0$ .

On the theoretical side, it would be useful to know the properties of a system of rods having a sloping repulsive potential and

a weak attractive force. This might best be done as a perturbation of the pure hard-rod solution of Onsager since this model seems to include the dominant features of rod ordering<sup>18</sup>. Brenner *et al.*<sup>40,41</sup> have made initial attempts at including a shallow square well potential. Quantitative estimates of rod spacing await a more refined statistical-mechanical theory.

A better explanation of ordering in TMV arrays is needed before reliable conclusions can be drawn on the questionable role of balancing forces among muscle proteins<sup>2,3,10-12,42-44</sup> or among proteins from other cells. We do not believe that long range protein attraction is a strong determinant of order in either viral or cellular protein arrays.

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# Reiteration frequency of the gene for tissue-specific histone H5 in the chicken genome

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*Chicken erythroid cells contain a tissue specific histone known as H5 in addition to the five major histone species found in other organisms. The mRNA coding for this histone has been isolated by indirect immunoprecipitation from immature, non-dividing reticulocytes in which this is the only histone synthesised. The mRNA has been modified by the enzymatic addition of a 3' polyadenylic acid tract, and transcribed into complementary DNA (cDNA) using the RNA-dependent DNA-polymerase from avian myeloblastosis virus. Studies on the hybridisation of this cDNA indicate that the gene coding for the H5 histone is reiterated 10 times in the chicken genome.*

ANALYSIS of the eukaryotic genome is difficult because of the complexity of the DNA and the presence of sequences which are repeated many times<sup>1,2</sup>. Nevertheless the kinetics of reassociation of pure mRNA, or its complementary DNA (cDNA) back to the total DNA of the genome, can be used to calculate the reiteration frequency of specific gene sequences. Such hybridisations have shown that many mRNAs, including those for globin<sup>3,4</sup>, silk fibroin<sup>5</sup>, and ovalbumin<sup>6</sup>, are transcribed from unique sequences in the DNA. It has been demonstrated that most of the mRNA in a variety of cells is transcribed from this 'unique' fraction of the DNA<sup>7-9</sup>. Two exceptions to this are the genes for feather keratins<sup>10</sup>, and histones<sup>11,12</sup> which are both found in the 'moderately repetitive' DNA. The histone genes in sea urchin embryos are present as tandemly repeated blocks

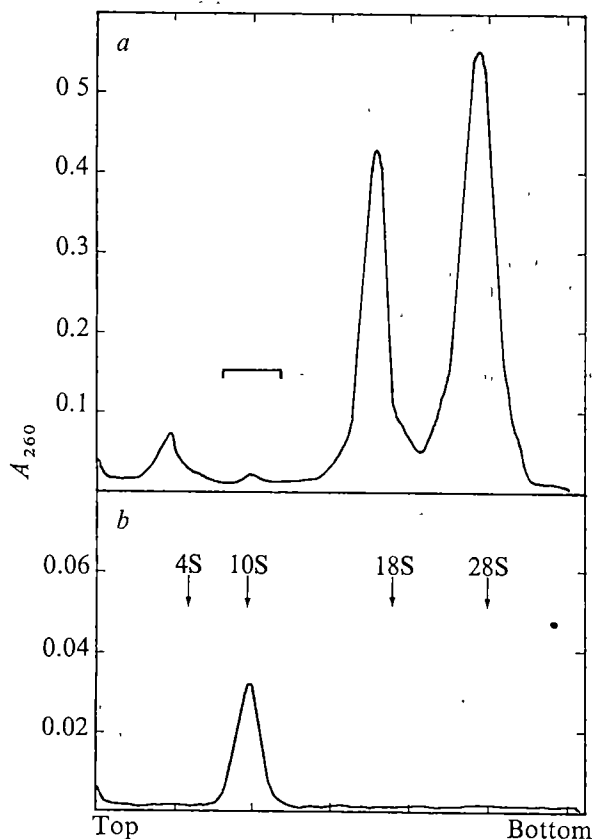
reiterated some 400-1,000 times (species variation) in the genome<sup>11,13</sup>. Cross hybridisation experiments indicate that the histone genes are also reiterated in the *Drosophila*<sup>14</sup> and *Xenopus*<sup>15</sup> genomes. Recent results show, however, that these genes are only reiterated some 10-20-fold in the genome of man<sup>15</sup> and mouse (quoted in ref. 15).

Histone gene reiteration frequencies have all been calculated from experiments in which highly labelled mRNA coding for all five histones was used as a probe. The nucleated red blood cells of birds, amphibians, and fish contain a sixth tissue-specific histone species known as H5 (ref. 16; the nomenclature is taken from reference 17). It has been suggested that this histone is involved in the final termination of transcription in these cells<sup>18</sup>. H5 is the only histone produced by the immature non-dividing reticulocyte<sup>19</sup> and its synthesis is therefore independent of DNA synthesis, unlike that of the other histones<sup>20,21</sup>. The mRNAs coding for these other histones are detectable only during S-phase and are rapidly turned over on cessation of DNA synthesis<sup>22,24</sup>. H5 mRNA on the other hand must be quite stable in the absence of DNA synthesis and thus subject to quite different controls. Whatever the biological significance of this, from a practical viewpoint it enables us to isolate the mRNA for a single histone species<sup>25</sup>. We report here the isolation of this mRNA, its transcription to produce cDNA, and the use of this cDNA to estimate the reiteration frequency of the gene coding for this highly unusual histone in the chicken genome.

## Isolation of H5 mRNA

Although H5 is the only histone produced by the avian reticulocyte<sup>19</sup>, the main protein product of this cell





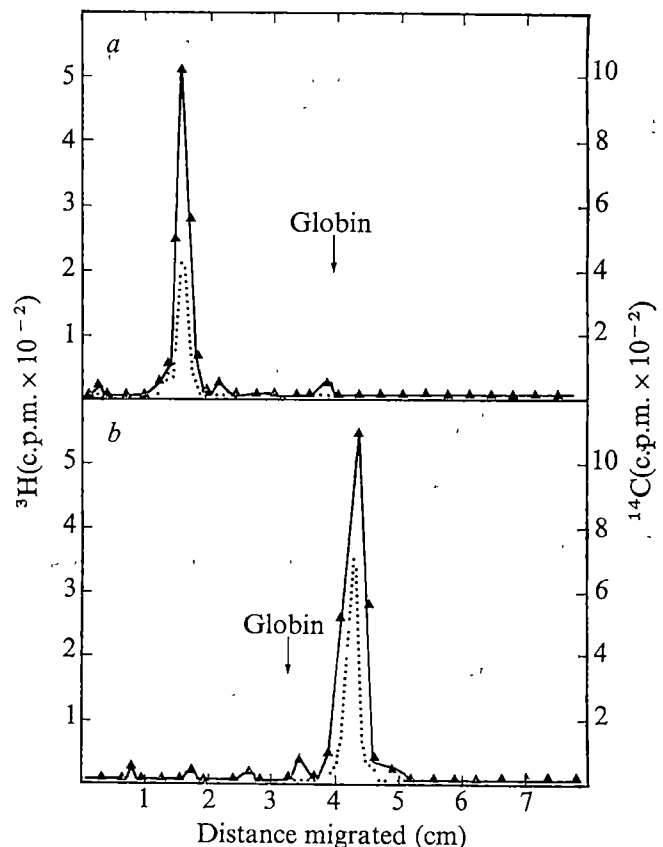
**Fig. 1** *a*, Profile at 260 nm of sucrose gradient containing RNA from H5-synthesising polysomes. Chicken polysomes were prepared as described<sup>25</sup> and diluted to 25  $A_{260}$  units  $\text{ml}^{-1}$  in 25 mM Tris (pH 7.6), 25 mM NaCl, 5 mM  $\text{MgCl}_2$ , 1  $\mu\text{g ml}^{-1}$  Trichodermin (Leo Pharmaceutical Products), 0.1  $\text{mg ml}^{-1}$  heparin. An aliquot (40  $\mu\text{g ml}^{-1}$ ) of rabbit anti-H5 (purified as described<sup>25</sup>) was added and the mixture incubated at 0 °C for 1 h. The antibody-polysome complexes were then precipitated by the addition of purified goat anti-rabbit immunoglobulin (1 mg per 30  $\mu\text{g}$  anti-H5). After 2 h at 0 °C (ref. 27) the precipitate was washed<sup>28</sup> to remove non specifically bound polysomes, and the RNA from the remaining polysomes was extracted with phenol<sup>29</sup>. This RNA was disaggregated in formamide, diluted, and centrifuged through a 10–40% (w/v) sucrose-sodium dodecyl sulphate (SDS) gradient<sup>25</sup> in a Beckman SW41 centrifuge rotor at 40,000 r.p.m. for 16 h. The fraction indicated by the bar was pooled and further purified as below. *b*, Profile at 260 nm of sucrose gradient containing purified H5 mRNA. The RNA described above was purified by 2 cycles of oligo(dT)-cellulose chromatography<sup>30</sup>, and the unbound RNA was then treated with formamide, diluted and centrifuged as above.

is globin<sup>25</sup>. Furthermore, since the expected size of the H5 mRNA is very close to that of globin mRNA<sup>25</sup>, it is impossible to use techniques based solely on the size of the mRNA to purify it. This has prompted the adoption of immunological methods to separate H5-producing polysomes. Indirect immunoprecipitation<sup>26</sup> is used to maximise the yield of H5 mRNA while minimising contamination with other mRNAs, especially globin mRNA. RNA was extracted from the polysomes<sup>29</sup>, and the 8–12S RNA separated on sucrose gradients (Fig. 1*a*). Polyadenylic acid-containing RNA was removed by two passages through oligo(dT)-cellulose and the unbound H5 mRNA repurified by further sucrose gradient fractionation. The 10S RNA thus produced (Fig 1*b*) did not act as a template for AMV RNA-dependent DNA-polymerase in conditions in which chicken globin mRNA was transcribed to 70% efficiency. This property, together with the lack of binding to oligo(dT)-cellulose, suggests that H5 mRNA is similar to other histone mRNAs in not having a 3' polyadenylic

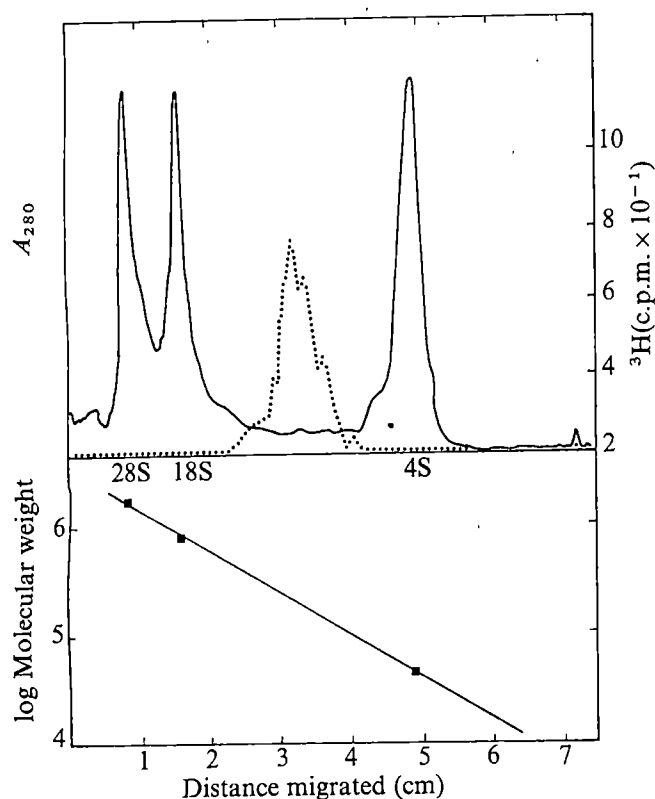
acid tract<sup>31,32</sup>. The H5 mRNA can be efficiently translated in the wheat embryo cell-free translation system to produce a single major product which coelectrophoreses with a  $^{14}\text{C}$ -labelled H5 standard, on two different polyacrylamide gel systems (Fig. 2). The 10S mRNA thus contains very little translatable globin mRNA and the only mRNA detectable is that coding for H5. This does not exclude the possibility that 10S-sized non-mRNA contaminants are present in the RNA however.

### Production and characterisation of H5 cDNA

In reactions which involve reassociation of trace amounts of a specific probe to a vast excess of total genomic DNA<sup>36</sup> it is essential that the probe is labelled to a high specific activity. This condition is fulfilled by the cDNAs produced from purified mRNA by AMV RNA-dependent DNA-polymerase, since highly labelled deoxynucleotide triphosphates are used as substrates<sup>37</sup>. A further advantage is that such cDNA probes do not suffer from the instability problems associated with RNA probes<sup>38</sup>. H5 mRNA was not, however, copied by the enzyme due to the absence of a 3' polyadenylic acid tract. This tract hybridises to short pieces of oligo(dT) which then act as a primer for the enzyme<sup>39</sup>. A short stretch of adenylic acid residues was therefore added to the 3' end of the mRNA, by an ATP-polynucleotidyltransferase prepared from maize<sup>40</sup>. The modified mRNA was then transcribed into cDNA and



**Fig. 2** Analysis on polyacrylamide gels of the translation products of H5 mRNA *in vitro*. H5 mRNA (0.5  $\mu\text{g}$ ) was translated in the wheat embryo cell-free system of Shih and Kaesberg<sup>33</sup> modified as described<sup>25</sup>. The labelled cell-free products were electrophoresed on *a* the SDS-urea gels of Swank and Munkres<sup>34</sup> and *b* the low pH urea gels of Panyun and Chalkley<sup>36</sup> as modified<sup>19</sup>. The gels were then sliced and radioactivity measured as described<sup>25</sup>.  $\blacktriangle$ — $\blacktriangle$   $^3\text{H}$ -labelled product of translation *in vitro*;  $\cdots$   $^{14}\text{C}$ -labelled H5 standard. Electrophoresis is from left to right in both cases. The  $^{14}\text{C}$  counts accounted for 10% spillover into the  $^3\text{H}$  channel and this was subtracted. The position of a  $^{14}\text{C}$  globin standard is indicated.



**Fig. 3** Estimation of the size of the cDNA produced from polyadenylated H5 mRNA. H5 mRNA (1  $\mu$ g) was incubated at 30 °C for 3 h in a 30- $\mu$ l incubation mix containing 70 mM Tris-acetate (pH 8.6), 10 mM dithiothreitol, 1 mM ATP, 1 mM  $MnCl_2$  and 50  $\mu$ g  $ml^{-1}$  of ATP-poly nucleotidyl transferase. The modified RNA was then ethanol precipitated and cDNA was synthesised from it, in the same tube, as described<sup>10</sup>. A portion of the purified cDNA was then electrophoresed on 4% polyacrylamide gels containing formamide<sup>47</sup>. 4S, 5S, 18S and 28S RNA were included as internal markers. Following electrophoresis the gels were scanned at 280 nm and then sliced and the pattern of radioactivity determined<sup>48</sup>.

formamide gel analysis of the product indicated an average length of 450 nucleotides (Fig. 3).

Although little other mRNA translational activity was detectable in the H5 mRNA preparation (Fig. 2), 10S-sized ribosomal RNA (rRNA) breakdown products have been described<sup>38</sup>. It is theoretically possible that the cDNA was preferentially copied from such contaminating RNA and it was therefore essential to prove that the cDNA made was complementary to H5 mRNA, and not to such contaminants. Since the two likely contaminating RNA species in the H5 mRNA preparation were rRNA and globin mRNA, this was readily tested by hybridising the cDNA to purified globin mRNA, 18S rRNA and 28S rRNA. The hybridisations were carried out in RNA excess and taken to a  $R_0t$  of 1 to ensure completion of the reaction. Hybrids were then assayed with S1 nuclease, a single-strand specific nuclease isolated from *Aspergillus*<sup>41</sup>. The data from such experiments allowed a direct estimate of the percentage of sequences in the cDNA preparation which were complementary to these different RNAs and are given in Table 1. The results show that H5 cDNA is contaminated only to about 3% with sequences complementary to rRNA (largely to 18S RNA) and a similar amount complementary to globin mRNA. If the globin mRNA used in these hybridisations had not, however, been purified by oligo(dT)-cellulose chromatography, then 85% of the H5 cDNA was protected from S1 degradation by the formation of hybrids (Table 1). This is merely indicative of the fact that chicken globin mRNA

**Table 1** Extent of cross hybridisation of H5 cDNA

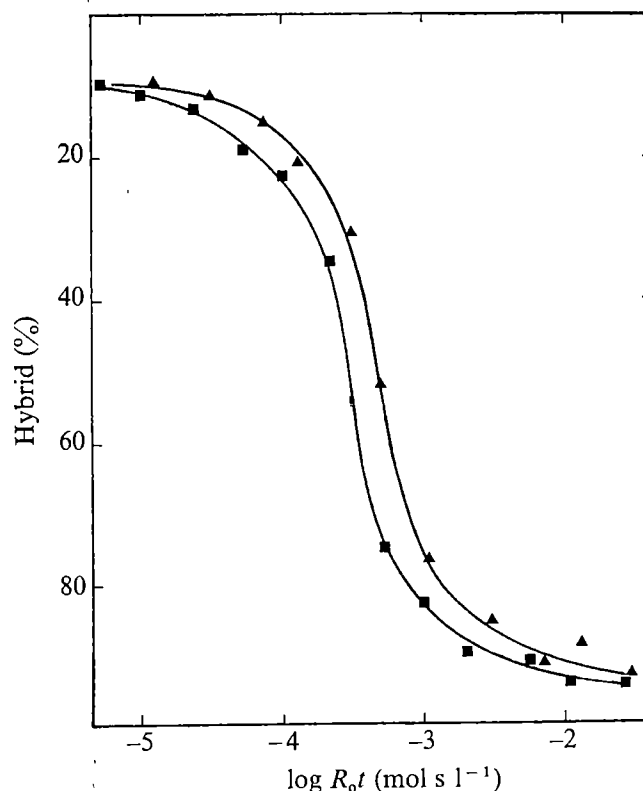
RNA added	$R_0t$ (mol s $l^{-1}$ )	Hybridisation (%)
18S rRNA	$6.8 \times 10^{-1}$	2.2
28S rRNA	$7.2 \times 10^{-1}$	1.0
'Crude' globin mRNA	$5.0 \times 10^{-1}$	85.4
'Purified' globin mRNA	$5.5 \times 10^{-1}$	3.0
H5 mRNA	$3.0 \times 10^{-1}$	89.0

H5 cDNA 2000 c.p.m. was hybridised to several electrophoretically pure RNA samples to the indicated  $R_0t$  values. The percentage of cDNA hybridised was then calculated by S1 nuclease assays. The percentage has been corrected by subtraction of the background due to S1-insensitive counts (about 4%). The 'crude' and 'purified' chicken globin mRNA refer to the same sample before and after purification by oligo(dT)-cellulose chromatography (see text).

preparations which have not been purified by oligo(dT)-cellulose chromatography<sup>42</sup>, contain a small amount of H5 mRNA. Because these hybridisations are done in a vast excess of RNA (compared with cDNA), sufficient of this contaminating H5 mRNA hybridises to protect 85% of the H5 cDNA from degradation by S1.

The kinetics and extent of the back-hybridisation of the H5 cDNA to excess, unlabelled H5 mRNA, can also provide some information about the fidelity of the copying of mRNA into cDNA. The kinetics of this hybridisation reaction are dependent on the sequence complexity of the mRNA and this can be estimated by comparison with a kinetic standard<sup>10</sup>. The standard used was the hybridisation of rabbit globin mRNA to its cDNA, and as determined by resistance of the hybrids to S1 nuclease, these hybridise in a sharp transition with a mid-point ( $R_0t_{1/2}$ ) of

**Fig. 4** Hybridisation of H5 mRNA and rabbit globin mRNA to their respective cDNAs. The reaction mixtures (50  $\mu$ l) contained 2,000 c.p.m. of H5 cDNA or 5,000 c.p.m. of globin cDNA, and varying amounts of mRNA. Hybridisation and S1 assays were as described<sup>10</sup>. ■—■, H5 mRNA-cDNA; ▲—▲, globin mRNA-cDNA.



$5 \times 10^{-4}$  (Fig. 4). This corresponds to a complexity of about 1,300 nucleotides for  $\alpha$  plus  $\beta$  globin mRNA, since it is known that cDNA probes to these two mRNAs do not cross hybridise to any appreciable extent<sup>13</sup>. In contrast, H5 mRNA hybridises to its cDNA with a similar sharp

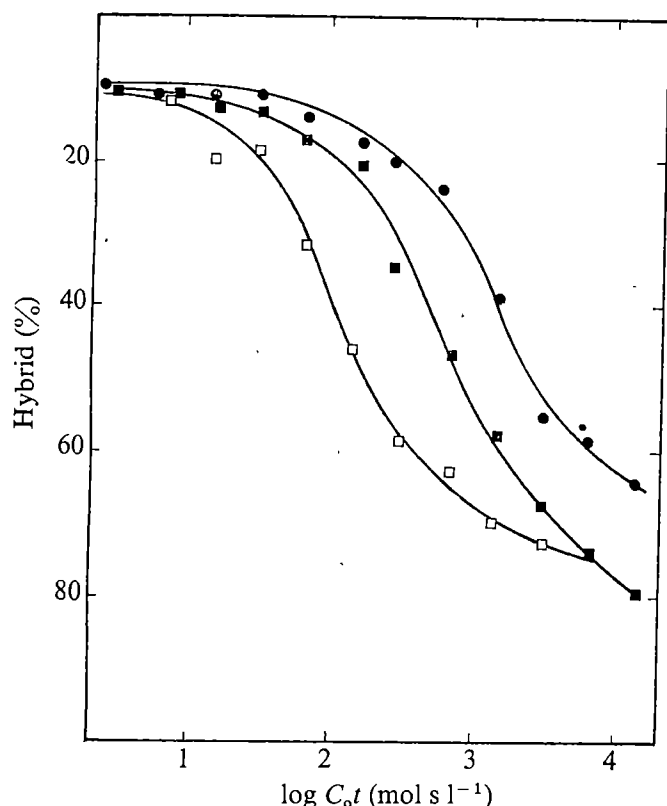


Fig. 5 Reassociation of H5 cDNA with excess chicken reticulocyte nuclear DNA. Three separate hybridisations were carried out in high salt buffer and the percent hybridisation assayed with nuclease S1 as described<sup>10</sup>. The three samples were: ●—●, H-chicken DNA-DNA; □—□, H5 cDNA-DNA; ■—■, chicken globin cDNA-DNA.

transition but with a  $R_{0.1/2}$  of about  $2.8 \times 10^{-4}$  (Fig. 4). This corresponds to a complexity of some 730 nucleotides which is the approximate size of the H5 mRNA (Fig. 1b). This indicates that H5 is coded by a single species of mRNA. The low  $R_{0.1/2}$  value and the sharpness of the transition also indicate that the H5 mRNA is not contaminated to any significant extent with other mRNAs.

### H5 gene reiteration frequency

The reiteration frequency of the H5 gene can be estimated by annealing the cDNA from H5 mRNA with a vast excess of total chicken DNA<sup>16</sup>. To determine the  $C_{0.1/2}$  for unique sequences, chicken globin cDNA was reannealed to chromosomal DNA and a mid-point ( $C_{0.1/2}$ ) of  $1.2 \times 10^2$  was obtained (Fig. 5). This is very similar to the  $C_{0.1/2}$  for the reassociation of total chicken DNA unique sequences (Fig. 5 and ref. 6), and is indicative of the fact that there are only 1 or 2 copies of the globin genes in the chicken genome. The hybridisation of H5 cDNA to chromosomal RNA, on the other hand, has a  $C_{0.1/2}$  of  $1.2 \times 10^2$  which means that the rate of the reaction is an order of magnitude faster than the unique rate. Since the globin and H5 cDNAs are of similar size and their G+C content is probably very similar (on the basis of the amino acid

analysis of the proteins), then this difference in rate must reflect a reiteration of the H5 gene in the chicken genome. The extent of reiteration is directly proportional to the  $C_{0.1/2}$  value compared with the kinetic standard<sup>16</sup>, and so the H5 gene is reiterated about 10 times. Furthermore, as the hybridisation goes to over 70% completion, the minor contaminating species in the H5 cDNA are not responsible for the reaction.

We have therefore demonstrated that the gene coding for H5 histone is reiterated in the chicken genome as are histone genes in all other organisms examined. The extent of reiteration is similar to that found in man<sup>15</sup> and mouse<sup>15</sup> but far less than is found in sea urchins<sup>11</sup>. The reason for the clustering of histone genes in sea urchins<sup>11,12,44</sup> is unknown but may be involved in the coordinate control of histone synthesis or in the linkage of histone mRNA synthesis to DNA synthesis<sup>23,45,46</sup>. Since H5 is subject to neither of these controls, and indeed is made only in red blood cells, the presence or absence of clustering of H5 genes with other histone genes may shed some light on this phenomenon. We are currently investigating the organisation of H5 histone genes in relation to other histone genes in the chicken genome.

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# Organisation of the genes coding for 5S RNA in the Chinese hamster

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*Hybridisation of iodinated mouse 5S RNA with Chinese hamster DNA fractionated on a CsCl gradient, identifies two separate DNA components that hybridise with the labelled probe. One component is slightly, and one considerably heavier than the main band DNA. This suggests that the gene coding for 5S RNA in the Chinese hamster may constitute two distinct populations differing in G+C content of their adjacent spacer regions.*

THE organisation of the genes coding for ribosomal 5S RNA in eukaryotes has been best studied in the frog *Xenopus*<sup>1,2</sup>. In this system the 5S genes occur as clusters of tandem repeats of alternating transcribed and non-transcribed nucleotide sequences which have been localised at the ends of most of the chromosomes by *in situ* hybridisation<sup>3</sup>. Analysis of the primary sequence of 5S ribosomal RNA (rRNA) has revealed two populations differing slightly in nucleotide sequence. One population is found predominantly in oocytes, the other in somatic cells<sup>4,5</sup>. In contrast to *Xenopus*, information regarding the organisation of the 5S genes in mammals is sparse. The organisation of these genes in the Chinese hamster was studied by examining their buoyant density characteristics in isopycnic CsCl gradients and here I report the results.

DNA from Chinese hamster V79 tissue culture cells was centrifuged to equilibrium in neutral CsCl. The gradients were fractionated and the DNA in each fraction was denatured and trapped on nitrocellulose filters as described previously<sup>6</sup>. To localise the 5S genes within the gradients, the DNA in each fraction was hybridised with <sup>125</sup>I-labelled 5S RNA obtained from a mouse liver ribosomal pellet. The mouse 5S RNA was prepared by gel filtration on a 90-cm Sephadex G-100 column and on acrylamide gel electrophoresis ran as a sharp band, well separated from a tRNA marker. Iodination was by the procedure described by Prenskey *et al.*<sup>7</sup>, yielding an RNA with specific activity of  $\sim 5 \times 10^6$ – $10^7$  c.p.m.  $\mu\text{g}^{-1}$ .

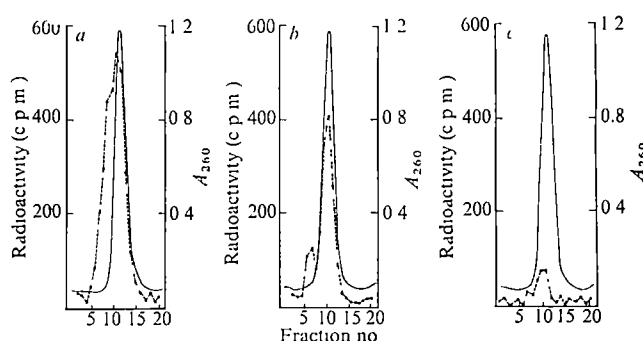
## Hybridisation with mouse 5S RNA

Mouse 5S RNA was selected as a probe for detecting the Chinese hamster 5S genes, so as to reduce the amount of hybridisation by RNAs contaminating the 5S RNA preparation. The nucleotide sequence of Chinese hamster 5S RNA (ref. 8) is virtually identical to that of other mammals examined<sup>9–12</sup> so mouse 5S RNA should hybridise faithfully to the Chinese hamster DNA, whereas contaminating RNA degradation products which are copurified with the 5S RNA, should cross hybridise poorly, if at all.

When the fractionated Chinese hamster DNA was hybridised with the mouse <sup>125</sup>I-5S RNA preparation, hybridisation occurred as a single broad peak with a shoulder on the heavy side of the DNA (Fig. 1a). The position of the radioactivity is reminiscent of the position at which the Chinese hamster ribosomal genes band in neutral CsCl<sup>6</sup>, suggesting that in addition to hybridisation of 5S RNA sequences, hybridisation of rRNA degradation

products which had been copurified with the 5S RNA, and which should also effectively cross hybridise with Chinese hamster DNA was also being detected.

To test the extent to which labelled ribosomal degradation products were contributing to the hybridisation shown in Fig. 1a, the DNA from fractions of a parallel gradient, which had been prepared at the same time, was hybridised with the same number of input counts of labelled mouse 5S RNA, but this time in the presence of an excess amount of unlabelled Chinese hamster rRNA (25  $\mu\text{g ml}^{-1}$ ). The results (Fig. 1b) show that about half of the hybridised radioactivity was taken out



**Fig 1** Hybridisation of iodinated mouse 5S RNA to Chinese hamster DNA. Chinese hamster DNA (50  $\mu\text{g}$ ) with a molecular weight of about  $10^7$  was fractionated in each of three identical CsCl gradients. The DNA of each fraction was denatured, trapped on nitrocellulose filters and hybridised with <sup>125</sup>I-mouse 5S RNA in the presence of the appropriate competing RNA. *a*, Chinese hamster DNA (50  $\mu\text{g}$ ) hybridised with a <sup>125</sup>I-mouse 5S RNA preparation ( $3 \times 10^6$  c.p.m. per 3 ml). *b*, Chinese hamster DNA (50  $\mu\text{g}$ ) hybridised with <sup>125</sup>I-mouse 5S RNA ( $3 \times 10^6$  c.p.m. per 3 ml) plus unlabelled Chinese hamster rRNA (25  $\mu\text{g ml}^{-1}$ ). *c*, Chinese hamster DNA (50  $\mu\text{g}$ ) hybridised with <sup>125</sup>I-mouse 5S RNA ( $3 \times 10^6$  c.p.m. per 3 ml) in the presence of unlabelled Chinese hamster rRNA (25  $\mu\text{g ml}^{-1}$ ) plus unlabelled *X. laevis* oocyte 5S RNA (20  $\mu\text{g ml}^{-1}$ ). —,  $A_{260}$ ; ●, radioactivity.

by competition, and that the radioactivity remaining resided in two discrete peaks, one considerably denser than the bulk of the DNA and the other only slightly heavier than the main band. To explore the possibility that these two peaks are due to hybridisation by sequences contained in 5S RNA, an additional competition hybridisation experiment was performed. The DNA in fractions from a third gradient, prepared at the same time as the previous two, was hybridised with the mouse <sup>125</sup>I-labelled 5S RNA preparation in the presence of unlabelled Chinese hamster rRNA (25  $\mu\text{g ml}^{-1}$ ) plus excess unlabelled 5S RNA (20  $\mu\text{g ml}^{-1}$ ) prepared from *Xenopus laevis* oocytes (a gift from Dr Ronald Brown). A 5S RNA competitor from such a distant evolutionary source was selected to reduce as much as possible the inclusion of RNAs that might contaminate the 5S RNA preparation and cross hybridise with the Chinese hamster DNA. If one of the radioactive peaks in Fig. 1b



represents hybridisation by a contaminating RNA in the mouse liver 5S RNA preparation, one would expect that in the presence of unlabelled rRNA and *Xenopus* oocyte 5S RNA one peak would disappear and the other, due to the contaminant, would remain intact. When the hybridisation reaction was performed in the presence of the two competing RNA populations, both peaks of radioactivity were virtually eliminated (Fig. 1b).

It is important to exclude any artefacts such as physical trapping of 5S DNA in the main band as the source of the hybridising material in the larger peak. To this end a series of four experiments, described in Fig. 2, was performed. Two 25-ml CsCl gradients each containing 1 mg of Chinese hamster DNA sheared to a molecular weight of about  $5 \times 10^6$  daltons were fractionated, and the heavy regions of the gradients containing the smaller peak of hybridisation were retained. The heavy region of one gradient was mixed with 100  $\mu$ g *Escherichia coli* DNA and that of the second gradient was mixed with 100  $\mu$ g Chinese hamster DNA. The DNA mixtures were banded in neutral CsCl, the gradients fractionated, and the DNA in each fraction hybridised with  $^{125}$ I-labelled mouse 5S RNA in the presence of a rRNA competitor. *E. coli* DNA has a 50% G+C content and as predicted bands at a density of 1.710 g cm $^{-3}$  (Fig. 2a). The peak of 5S RNA hybridisation occurs at a density of 1.718 with no significant hybridisation associated with the bulk of the *E. coli* DNA, indicating that physical trapping of 5S DNA sequences in the gradient has not occurred.

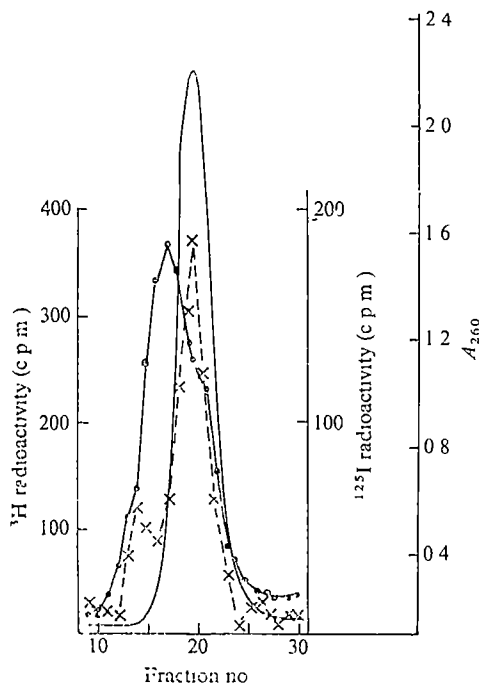


Fig. 2 Hybridisation to Chinese hamster ribosomal and 5S genes in a neutral CsCl gradient. A CsCl gradient containing 100  $\mu$ g Chinese hamster DNA was fractionated and DNA from each fraction was denatured and adsorbed on to nitrocellulose filters. The filters were cut in half and one set of filter halves was hybridised with  $^{125}$ I-mouse 5S RNA in the presence of unlabelled Chinese hamster rRNA (25  $\mu$ g ml $^{-1}$ ). The second set of filter halves was hybridised with  $^3$ H-ribosomal cRNA from *X. laevis* (provided by Dr Donald D. Brown). The *Xenopus* ribosomal cRNA hybridises specifically with sequences complementary to 18S and 28S RNA within the Chinese hamster genome<sup>6</sup>. The results described in Fig. 3 show that the ribosomal genes band directly between the two peaks of radioactivity generated by hybridisation of the mouse  $^{125}$ I-5S RNA preparation, thus excluding hybridisation by rRNA sequences as a source for either of the peaks. These results are also consistent with the observation that Chinese hamster rDNA has a buoyant density of 1.708 as determined by analytical ultracentrifugation of a partially purified rDNA preparation (unpublished data).

When *E. coli* DNA alone was banded in CsCl and hybridised with  $^{125}$ I-labelled 5S RNA, no radioactivity was retained on the filters (Fig. 2b), eliminating the exchange of radioactive iodine from the RNA probe to the DNA on the filters as the

source of the larger peak of radioactivity. When Chinese hamster DNA, containing the additional heavy DNA from the larger gradient, was banded in CsCl and hybridised, the bulk DNA banded as expected at a density of 1.700 (Fig. 2c). Hybridisation results in two peaks of radioactivity at 1.718 and 1.702, with the heavier peak substantially enriched. The important observation is that both radioactive peaks band faithfully at their predicted densities. In a situation where the region of a gradient containing the heavy peak has been removed and the DNA is rebanded in CsCl, only one peak of radioactivity at a density of 1.702 is revealed on subsequent fractionation and hybridisation (Fig. 2d). The combined results of these experiments confirm that the two peaks of radioactivity obtained on hybridisation with  $^{125}$ I-labelled 5S RNA reflect true populations of DNA that band faithfully at densities of 1.718 and 1.702 respectively.

The possibility remains that one of the two radioactive peaks is due to residual hybridisation of labelled rRNA degradation products and that the added *Xenopus* 5S RNA in Fig. 1c contains sufficient rRNA sequences to complete the competition. This interpretation was eliminated by an experiment in which the DNA from fractions of a gradient was trapped on nitrocellulose filters and the filters cut in half. One set of filter halves was hybridised with mouse  $^{125}$ I-labelled 5S RNA in the presence of excess unlabelled rRNA and the other set of filter halves was hybridised with a ribosomal  $^3$ H complementary RNA (cRNA) synthesised from purified *Xenopus* rDNA (provided by Dr Donald D. Brown). The *Xenopus* ribosomal cRNA hybridises specifically with sequences complementary to 18S and 28S RNA within the Chinese hamster genome<sup>6</sup>. The results described in Fig. 3 show that the ribosomal genes band directly between the two peaks of radioactivity generated by hybridisation of the mouse  $^{125}$ I-5S RNA preparation, thus excluding hybridisation by rRNA sequences as a source for either of the peaks. These results are also consistent with the observation that Chinese hamster rDNA has a buoyant density of 1.708 as determined by analytical ultracentrifugation of a partially purified rDNA preparation (unpublished data).

## Organisation of 5S RNA genes

The data obtained from these experiments allow three interpretations. The first is that a mouse liver polysomal pellet and *Xenopus laevis* oocytes contain an RNA in common that is neither 5S RNA, rRNA nor tRNA, and which hybridises effectively with Chinese hamster DNA. Although unlikely, this possibility has not, as yet, been completely excluded.

A second interpretation would argue that 5S genes occur as a series of clusters within blocks of high G+C non-5S DNA. The rationale is that two peaks of hybridisation can be generated by random breakage of DNA which would result in pure 5S DNA molecules from the internal region of the cluster and a small fraction of 5S DNA molecules containing varying amounts of the external high G+C non-5S DNA. Such a distribution seems unlikely for at least two reasons. First, one would predict that as the DNA is sheared to a smaller size the amount of radioactivity contributed by the smaller peak should decrease, yet the ratio of small peak to large peak remains relatively constant for DNAs between  $5 \times 10^6$  and  $2 \times 10^7$  daltons. When the DNA is further sheared to between  $5 \times 10^5$  and  $7 \times 10^5$  daltons, hybridisation occurs as a single broad band with a peak of radioactivity at about 1.715 g cm $^{-3}$  (unpublished). These data, however, cannot distinguish between a single component comprising a broad band with a peak at 1.715 g cm $^{-3}$  and two components of different buoyant density that cannot be resolved. Second, since the fragments containing the external regions should be heterogeneous with respect to their content of high G+C DNA, one would expect such fragments to band as a broad smear in the heavy part of the gradient. In fact, however, the smaller peak of radioactivity bands sharply,

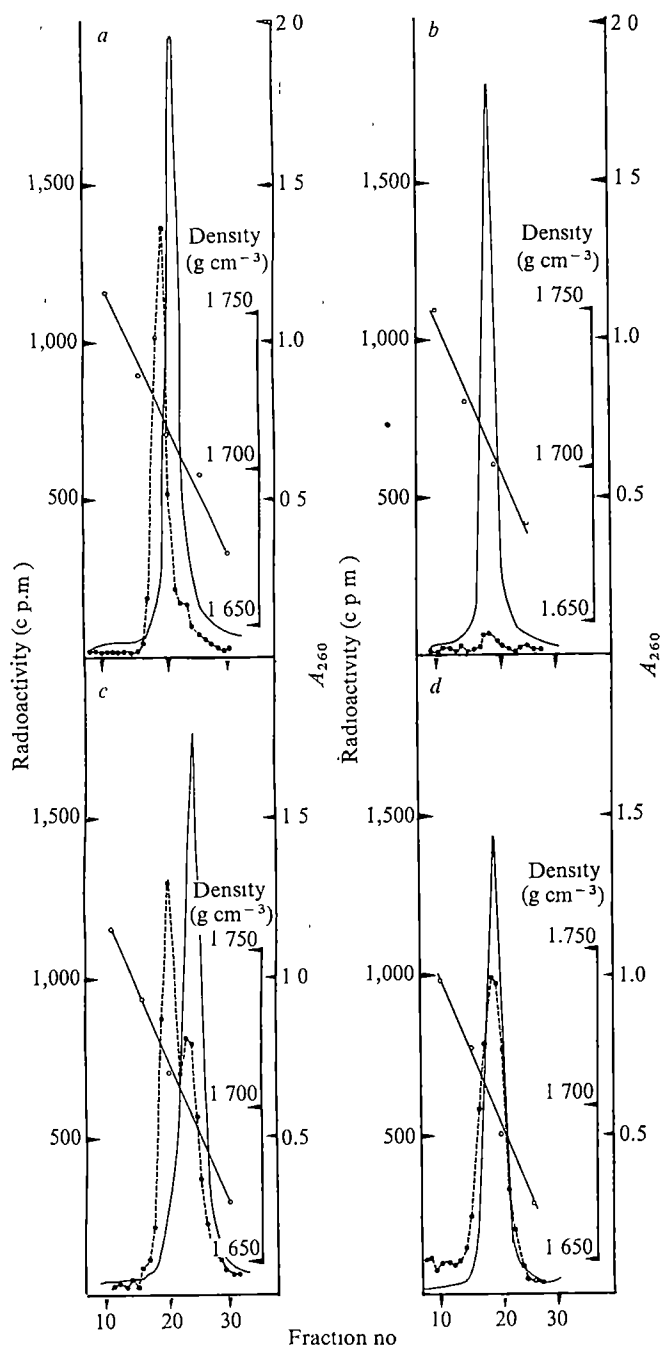


Fig. 3 Rebanding of the DNA hybridising with  $^{125}\text{I}$ -5S RNA. *a*, Chinese hamster DNA banding at  $1.718\text{ g cm}^{-3}$  from a 25-ml  $\text{CsCl}$  gradient containing 1 mg DNA (sheared to  $5 \times 10^6$  daltons) was added to 100  $\mu\text{g}$  *E. coli* DNA (about  $10^7$  daltons). This DNA mixture was recentrifuged in neutral  $\text{CsCl}$ , the gradient was fractionated, and the DNA was hybridised with mouse  $^{125}\text{I}$ -5S RNA in the presence of rRNA competitor. *b*, *E. coli* DNA was banded in neutral  $\text{CsCl}$ , the gradient was fractionated, and the DNA in each fraction was hybridised with mouse  $^{125}\text{I}$ -5S RNA in the presence of a rRNA competitor. *c*, Chinese hamster DNA banding at  $1.718\text{ g cm}^{-3}$  from a 25-ml  $\text{CsCl}$  gradient containing 1 mg DNA ( $5 \times 10^6$  daltons) was added to 100  $\mu\text{g}$  Chinese hamster DNA ( $5 \times 10^6$ ) and recentrifuged in  $\text{CsCl}$ . The gradient was fractionated, the DNA hybridised with mouse  $^{125}\text{I}$ -5S RNA in the presence of a rRNA competitor. *d*, 100  $\mu\text{g}$  Chinese hamster DNA was centrifuged in neutral  $\text{CsCl}$  and the region banding at  $1.718\text{ g cm}^{-3}$  was removed. The remaining DNA was recentrifuged in  $\text{CsCl}$ , the gradient was fractionated and the DNA in each fraction hybridised with mouse  $^{125}\text{I}$ -5S RNA in the presence of a rRNA competitor. All gradients were centrifuged at the same time in a Beckman 50 Ti rotor. The filters containing the denatured DNA from all four gradients were incubated together in a reaction mixture containing 50% formamide,  $4 \times \text{SSC}$ , Chinese hamster rRNA ( $25\text{ }\mu\text{g ml}^{-1}$ ) and mouse  $^{125}\text{I}$ -5S RNA ( $1.2 \times 10^6\text{ c.p.m. per } 12\text{ ml}$ ) at  $37^\circ\text{C}$  for 30 h. The density profile of each gradient was determined from the refractive index of a 4- $\mu\text{l}$  aliquot from every fifth fraction. —  $A_{260}$ ; ---, radioactivity; O—O, density.

multiple populations of 5S genes since such heterogeneity has already been demonstrated in *X. laevis*<sup>4,5</sup>, and since in human cells, 5S genes may be scattered in a large number of chromosomes<sup>13</sup>. In HeLa cells, molecular hybridisation studies with fractionated chromosomes have suggested that the 5S genes numbering about 2,000 per haploid genome<sup>14</sup> are contained on chromosomes of all size classes with some enrichment in the largest size group<sup>13</sup>. *In situ* hybridisation with chromosomes of human diploid cells has suggested that a cluster of 5S genes is located on chromosome 1, although scattered grains were also observable over many of the other chromosomes<sup>15,16</sup>. In *X. laevis*, two gene populations were detected by differences in the primary sequence of the 5S RNAs themselves<sup>4,5</sup> rather than by differences in their spacer DNAs. Genes coding for 5S RNA have been isolated from both *X. laevis* (1) and *X. mulleri* (2) and in both cases, the spacer regions constitute most of the 5S DNA. Since the transcribed regions of *X. laevis* and *X. mulleri* 5S DNA comprise only 6 and 14% respectively of the 5S DNA<sup>2</sup>, the behaviour of this DNA in isopycnic gradients depends more on the base composition of the spacer than the transcribed region. If the same relationship between spacer and transcribed DNA exists in the 5S DNA of mammals, subpopulations of this gene differing primarily in the base composition of the spacer should be detectable in isopycnic gradients and should yield similar results to those presented in these experiments.

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indicating a relatively homogeneous population of molecules.

A third interpretation of the results is that the Chinese hamster genome contains two populations of genes that code for 5S RNA but which differ in the G+C content of their adjacent spacer regions. Such differences in base composition of the spacer would confer different buoyant densities on the two gene populations in  $\text{CsCl}$  and would result in the two peaks of radioactivity generated by hybridisation. Since the G+C content of mammalian 5S RNA is 60% (refs 9–12), the corresponding DNA should have a buoyant density of  $1.720\text{ g cm}^{-3}$ . The lighter population bands at  $1.702\text{ g cm}^{-3}$  and should therefore have an A+T-rich spacer. The smaller and heavier population bands at  $1.718\text{ g cm}^{-3}$  and would therefore be predicted to contain a spacer with a base composition much like that of the transcribed region. Unequivocal identification of both peaks of radioactivity as 5S genes will require the demonstration that the RNAs hybridising in each peak are 5S RNA, and experiments to that end are currently in progress.

It is not unreasonable to expect mammalian cells to contain

# letters to nature

## Origin of the black hole in Cyg X-1

THE evidence that Cygnus X-1 is a black hole of mass  $\gtrsim 9M_{\odot}$  (ref. 1) is not conclusive, but is sufficient to warrant some consideration of the implications of such an object for the late stages of stellar evolution. We argue here that such a black hole must form directly by implosion of a star of mass  $\gtrsim 30M_{\odot}$  rather than by accretion on to a neutron star. The Galaxy may contain  $\sim 10^7$  such massive black holes.

Supernova models based on either the degenerate cores of intermediate mass stars ( $4-8M_{\odot}$ , see ref. 2) or the iron cores of more massive stars<sup>3</sup>, suggest a collapsing core of initial mass  $\sim 1.4M_{\odot}$  and hence a final gravitational mass of  $\sim 1.2M_{\odot}$ . In the more massive stars the collapse of some material of lower atomic weight than iron might increase the final mass somewhat (W. D. Arnett, private communication). The estimated masses for the X-ray sources Her X-1 ( $\sim 1.3M_{\odot}$ , A. B. Middleton and C. D. Nelson, unpublished), Cen X-3 ( $0.6-1.1M_{\odot}$ , ref. 1), and Vela X-1 ( $1.6-2.1M_{\odot}$ , refs 4, 5), which are almost certainly neutron stars because of their X-ray pulsations, accord well with these estimates and with recent calculations of the maximum mass of neutron stars  $M_{\text{lim}} \sim 1.5M_{\odot}$  (ref. 6) to  $2.4M_{\odot}$  (ref. 7). For sources without periodic X-ray variations, the mass estimates are less certain; however, with the exception of Cyg X-1, the estimated masses cluster  $\sim 1-2M_{\odot}$  (see Table 1). In summary, all known binary X-ray sources other than Cyg X-1 have masses consistent with those expected for neutron stars somewhat below  $M_{\text{lim}}$  or black holes that are only slightly above  $M_{\text{lim}}$ .

One might envisage two alternative origins for a hole  $\gtrsim 9M_{\odot}$  in Cyg X-1: either it was formed at essentially its present mass by the implosion of a massive star, or it began as a neutron star that grew to its present mass through accretion, during which growth it collapsed into a black hole when it passed  $M_{\text{lim}}$ . We first discuss the second possibility.

The time scale for growth by accretion is restricted by the Eddington limit. For

$$L_X \leq L_{\text{Edd}} = 4\pi c G m_{\text{H}} M / \sigma_T,$$

where  $\sigma_T$  is the Thomson cross section, we have

$$\tau_{\text{grow}} \equiv M/\dot{M} \geq \frac{c\sigma_T}{4\pi G m_{\text{H}}} f = 1.1 \times 10^8 f_* \text{ yr} \quad (1)$$

Here  $f \equiv L/\dot{M}c^2$  and  $f_* \equiv f/0.25$ . Since many X-ray sources seem to radiate at nearly the Eddington limit, it is reasonable to assume that  $\tau_{\text{grow}}$  will be near the lower limit given by equation (1). For black holes, we take  $f_* = 1$  because expected efficiencies are  $f = 0.42$  and  $0.06$  for maximally rotating and

non-rotating holes, respectively<sup>10</sup>. Therefore  $\tau_{\text{BH}} \gtrsim 1.1 \times 10^8 \text{ yr}$ . Since the companion of Cyg X-1 (HDE 226868) has a main sequence lifetime  $\lesssim 5 \times 10^8 \text{ yr}$  for its inferred mass  $\gtrsim 25M_{\odot}$  (ref. 1), Cyg X-1 cannot have grown from  $M_{\text{lim}} \sim 2M_{\odot}$  to  $\gtrsim 9M_{\odot}$  unless  $f_* \lesssim 0.05$ . There is no basis for assuming such a low efficiency, and we conclude that the black hole in Cyg X-1 formed, as such, at nearly its present mass.

Our conclusion, that the black hole of  $\sim 10M_{\odot}$  in Cyg X-1 was formed directly in a stellar implosion, is somewhat surprising because recent work on stellar evolution has indicated that even very massive stars may form only neutron stars of  $M \sim 1.2M_{\odot}$ . Studies by Arnett<sup>3</sup> suggest that massive stars end their lives with the collapse of an iron core of  $M \sim 1.4M_{\odot}$ , regardless of the total mass of the star. Most of these stars must explode leaving only a neutron star remnant, according to arguments involving the abundances of carbon and oxygen in the Universe.

Stars with  $M \gtrsim 10M_{\odot}$  contain layers of carbon and oxygen which can potentially be ejected. By contrast, stellar evolution implies that intermediate and low mass stars can eject no carbon and oxygen; they either trap these elements in white dwarf remnants or convert them to iron peak elements in the process of core collapse and/or explosion. To provide enough carbon and oxygen to the interstellar gas, most stars with  $M \gtrsim 10M_{\odot}$  must eject their CO layers rather than suffer a total collapse. Since the iron cores are nearly identical and their evolution and dynamics seem to be little affected by the total stellar mass, a tempting generalisation is to assume that all massive stars explode with the formation of a neutron star from the iron core.

The creation of a black hole in Cyg X-1 with an initial mass  $\gtrsim 9M_{\odot}$  indicates that this generalisation is not correct. In spite of the homogeneity of Arnett's pre-collapse configurations, the direct formation of a black hole of  $\sim 10M_{\odot}$  implies that an unknown factor, presumably associated with the increasing mass of the post-helium burning core, becomes important for sufficiently large stellar masses. This factor evidently alters either the pre-collapse configuration or the dynamics of the explosion.

A mass of  $10M_{\odot}$  for the black hole is consistent with this picture. One expects the hydrogen envelope to have been transferred from the star which made the black hole to HDE226868 in the course of evolution, so that the object which finally collapsed was a bare core. A core of  $\sim 10M_{\odot}$  would correspond to an initial main sequence mass of  $\sim 30M_{\odot}$  (ref. 3) for the star which formed the black hole. The assumption that HDE226868 now has  $M = 30M_{\odot}$  implies that it originally had a mass of  $\sim 10M_{\odot}$ .

According to Ostriker *et al.*<sup>11</sup>, the local density of stars  $\gtrsim 30M_{\odot}$  is  $\sim 2 \text{ kpc}^{-2}$ . If all such stars make black holes directly and one in ten occurs in a close binary, the density of such systems is consistent with the distance  $\sim 2 \text{ kpc}$  to Cyg X-1 (ref. 12). Many systems would spend their entire main sequence lives obscured within the dense clouds that gave them birth. Over its lifetime, the Galaxy would have produced  $\sim 10^7$  massive black holes.

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Table 1 Estimated masses of binary X-ray sources

Name	Mass $M/M_{\odot}$	Ref.
3U1956+35 Cyg X-1	$\geq 9$	1
1118-60 Cen X-3	0.6-1.1	1
1653+35 Her X-1	1.3	*
0900-40 Vela X-1	1.6-2.1	5
1700-37	$1.3 \pm 0.2$	8
0115-37 SMC X-1	$\sim 2$	8
1617-15 Sco X-1	$< 2$	9
2030+40 Cyg X-3	$\sim 1(?)$	12
2142+38 Cyg X-2	$\sim 1(?)$	12

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## Production of heavy elements in nature

We shall describe here a possible mechanism for the production of heavy elements (with  $A > 60$ ): these elements, we suggest, may be formed in the matter ejected from the envelope of a neutron star during the starquake process.

The  $r$ -process (rapid neutron capture) is known to be capable of forming heavy elements, and is believed to take place during a supernova outburst<sup>1</sup>. Calculations<sup>2,3</sup> of a supernova outburst show, however, that the neutron fluxes might be too low to explain the synthesis of heavy elements. Because of this, neutron stars are, in our opinion, among the most promising sites for the nucleosynthesis of heavy elements.

The mechanism of heavy element formation by the fission of the even heavier elements which can exist in neutron-rich nuclear fluid, was proposed by Goepfert-Mayer and Teller<sup>4</sup> in 1947. The discovery of pulsars and their interpretation as neutron stars has provided the ideal initial conditions for the Mayer-Teller model<sup>5,6</sup>. The existence of heavy neutron-rich nuclei was discussed by Zel'dovich<sup>7</sup> and by Brueckner<sup>8</sup>, who considered neutron droplets in conditions of high pressure. Afterwards the chemical composition of neutron-star envelopes at densities  $\rho = 10^{10}$ - $10^{12}$  g cm<sup>-3</sup> was considered<sup>6,9,10</sup>. In such conditions they are made of nuclei with  $A \sim 200$ -500 and free neutrons.

We consider the case where instabilities cause the ejection of matter from the neutron star surface. It will be shown that the evolution of ejected matter ends with the formation of heavy (even superheavy) elements<sup>11</sup>. By contrast with ref. 4, matter composed of neutron-rich nuclei rather than of neutrons only will be considered. The heavy nuclei obtained in this way will be ejected from the magnetosphere of a neutron star, and may enrich interstellar space<sup>12</sup>.

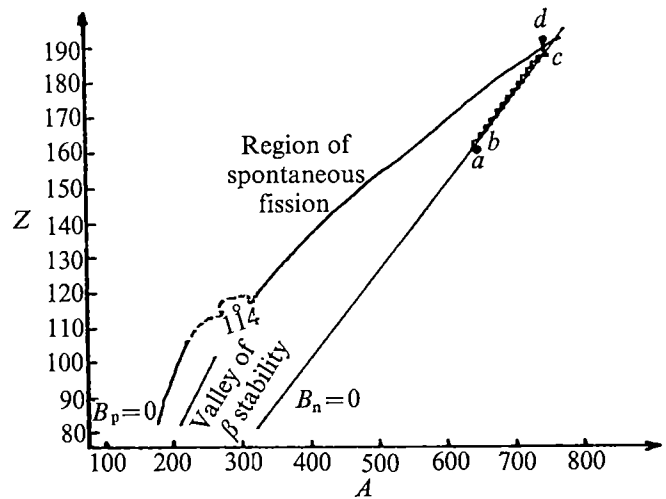
Initially we have nuclei with  $(Z, A)$ , as shown in Fig. 1, point  $a$ . The chemical potential of electrons decreases as the matter expands,

$$\mu_e \sim mc^2 \left( \frac{\rho}{\mu_o \times 10^6} \right)^{1/3}$$

where  $\mu_o$  is the molecular weight of electrons, and is given by

$$\mu_o = \frac{\sum n_i A_i}{\sum n_i Z_i}$$

The  $(Z, A)$  nuclei decay by  $\beta$  emission and the new nuclei  $(Z+1, A)$  will capture free neutrons. We assume that on the boundary of stability, the relation  $Z/A = 1/4$  holds.



Capture of four neutrons

$$\beta \text{ decay } a(Z, A) \rightarrow b(Z+1, A+4)$$

$$(t_{sf} \approx 0.01 \text{ s})$$

$$B_n = 0$$

Fig. 1 Stability of superheavy nuclei. A nucleus at point  $a$  moves by  $\beta$  decay, and capture of neutrons (see inset) to point  $d$ , where it undergoes fission.

For the original nuclei at  $\rho = 10^{10}$  g cm<sup>-3</sup> we have  $Z = 161$ ,  $A = 644$  according to ref. 6. So, the  $\beta$  decay of the  $(Z, A)$  nuclei will be followed by four neutron captures by nuclei  $(Z+1, A)$ —point  $b$  of Fig. 1. The following conditions on the characteristic times will be satisfied:  $t_h \leq t_\beta$ ,  $t_\beta > t_n$ , where  $t_h$ ,  $t_\beta$ , and  $t_n$  are the time scales of hydrodynamic expansion,  $\beta$  decay and  $(n, \gamma)$  reactions, respectively. These time scales are defined by:  $t_h = 446a/\sqrt{\rho}$  in seconds, where  $a$  is an arbitrary scaling parameter,

$$t_n = \frac{m_H}{\alpha \rho \langle \sigma v \rangle}$$

where  $m_H = 1.67 \times 10^{-24}$  g,  $\alpha$  is the partial weight of neutrons in the matter,

$$t_\beta = \frac{6\Delta}{10^{-4} W_o^6}$$

where  $\Delta$  is the density of nuclear states (in all cases we assume  $\Delta = 15$ ) and  $W_o$  is the nuclear mass difference. We use here the notation introduced in ref. 1. For matter in these conditions, the neutron capture cross section  $\sigma \sim 10^{-25}$  cm<sup>2</sup>, mean velocity of neutrons  $v \sim 10^8$  cm s<sup>-1</sup>, so

$$t_n = \frac{10^{-7}}{\alpha \rho}$$

The calculated  $t_\beta$  values are given in Table 1. The characteristic time  $t_\beta$  does not depend on density, and  $t_n$  is inversely proportional to density. Nuclei moving along their 'evolutionary'

Table 1 Half lives for  $\beta$  decay of extremely heavy elements in seconds

	160	161	162	163	164	165	166	167	168	169	170	171	172
$A$	640	644	648	652	656	660	664	668	672	676	680	684	688
$t_\beta$	0.011	0.011	0.011	0.012	0.012	0.012	0.013	0.013	0.014	0.014	0.015	0.015	0.016
$Z$	173	174	175	176	177	178	179	180	181	182	183	184	185
$A$	692	696	700	704	708	712	716	720	724	728	732	736	740
$t_\beta$	0.016	0.017	0.018	0.018	0.019	0.020	0.020	0.021	0.022	0.023	0.024	0.025	0.025



Table 2 Logarithms of spontaneous fission half lives (for  $Z = 184$ ,  $t_{sf} = 0.014$  s)

$Z$	160	161	162	163	164	—	181	182	183	184	185	186
$A$	640	644	648	652	656	—	724	728	732	736	740	744
$\lg(t_{sf})$	18.073	19.899	13.697	18.486	12.284	—	5.7685	-0.433	-4.3555	-1.8460	2.9425	-3.2590

path increase their atomic number  $Z$  and approach the region of spontaneous-fission instability. The spontaneous-fission half lives ( $t_{sf}$ ) were calculated using the formula<sup>13</sup>

$$t_{sf} = 10^{-21} \times 10^{7.85 E_b(s)}$$

where

$$E_b = 19.0 - 0.36Z^2/A + \varepsilon \text{ (MeV)}$$

is the fission barrier,  $\varepsilon = 0.7$  for odd-odd nuclei,  $\varepsilon = 0.4$  for odd- $A$  nuclei, and  $\varepsilon = 0$  for even-even nuclei. Calculated half lives are given in Table 2. When nuclei reach the fission region (point  $d$  on Fig. 1) multiple fission might occur (triple or even more). Fission fragments will capture neutrons and some  $r$ -process-like recycling will take place.

Calculations on this model lead to the following picture. Within 0.4 s after the ejection of matter (at  $t_0$ ) from the star, more than half the original nuclei with  $Z = 161$ ,  $A = 644$  move on their evolutionary tracks up to the point with  $Z = 184$ ,  $A = 736$ , where spontaneous fission occurs. Half a second after the initial ejection, almost all the nuclei have done this. The density of matter at  $t = t_0 + 0.4$  s and  $t = t_0 + 0.5$  s is  $5 \times 10^6$  and  $3 \times 10^6$  g cm<sup>-3</sup>, respectively, and the characteristic times  $t_n$  are  $2 \times 10^{-14}$  and  $3 \times 10^{-14}$  s, respectively, so that  $t_n < t_\beta$ .

Both the fission fragments and the products of  $\beta$  decay can be in excited states. A probable means of de-excitation of these nuclei may be the evaporation of some neutrons, leading to the formation of neutron-deficient nuclei. The probability for such process is low but the abundance of neutron-deficient nuclei amounts to  $10^{-4}$ – $10^{-5}$  of that of heavy nuclei<sup>14</sup>.

The described picture is in a good agreement with an estimate of the number of neutron stars in the Galaxy. To obtain the observed density of heavy elements, we need  $10^9$  neutron stars assuming that 10% of their envelopes form heavy elements and are ejected from the stars. In this way the neutron star may enrich with heavy elements the surfaces of stars in its neighbourhood. This mechanism may explain the presence of promethium on the surface of some stars. The longest lived isotope of promethium is <sup>145</sup>Pm with  $t_{1/2} = 17.7$  yr. Lines of Pm have been found<sup>15,16</sup> in the spectra of some Ap stars, but confirmation is needed. Another prediction of this model is the correlation between the  $\gamma$ -ray (of energy 2 keV–10 MeV) and heavy elements outburst.

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## Lunar effect in the quiet-time $D_{st}$ index

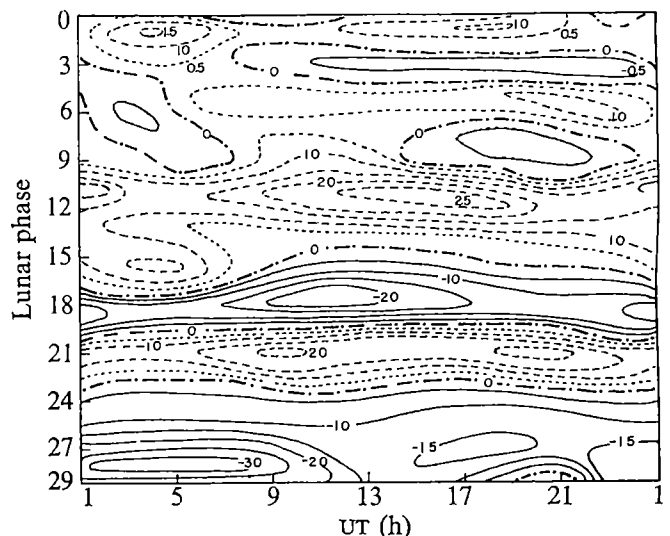
POLARISATION electric fields produced in the dynamo region should, theoretically, have a considerable effect on the distribution and energy of magnetospheric particles, but none has been found previously<sup>1–3</sup>. Here we show that the  $D_{st}$  index calculated by Sugiura and Poros<sup>4</sup> shows a semidiurnal variation, which we attribute to the effect of tides (caused by the Moon) in the dynamo region, thus verifying the theory.

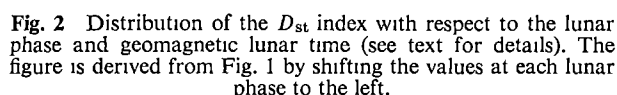
The data used are the hourly values of the  $D_{st}$  index on five international quiet days in each month for 14 years (1959–72), the total number of data points being 21,000 ( $25 \times 5 \times 12 \times 14$ ). These values are obtained for each hour of universal time (UT), and are dependent on the month of the year and also on the lunar phase. We have checked carefully that the data were collected uniformly over the lunar phases.

We have found a semiannual variation in the  $D_{st}$  index which will be discussed elsewhere. Our discussion is here limited to the relation of the  $D_{st}$  index to UT and to the lunar phase, so that we have used the data averaged over the whole month and whole year.

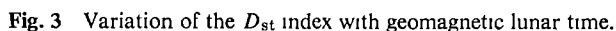
Figure 1 shows the distribution of  $D_{st}$  values with respect to UT and lunar phase. It is found that the  $D_{st}$  values show a systematic change with lunar phase, such that the positive and negative values appear alternately. To see more, the  $D_{st}$  values are rearranged in 'geomagnetic lunar time', that is, in hours measured from the lower transit of the Moon across the meridian containing the north magnetic pole. This is shown in Fig. 2, where the abscissa shows the lunar time relating to the geomagnetic axis: at 0 h the geomag-

Fig. 1 Distribution of the  $D_{st}$  index with respect to the lunar phase and UT (in units of nT).





Although the  $D_{\text{st}}$  index derived from geomagnetic variations on the ground may include various effects, the main contribution seems to come from the ring current. We



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## Evolution of periodicities exhibited by fluctuations in the intensity of chorus

Here, the power spectral analysis method<sup>4,5</sup> is used to investigate, quantitatively, the evolution of the periodicities exhibited by the intensity fluctuations of a 60-min recording of chorus. The recording was made at St John's Stadan Station, Newfoundland, Canada ( $L=3.5$ ) on March 7, 1970, at 0850 UT. The recording was started close to the peak of an intense chorus event which lasted for more than 2 h. The short (2–3 s) period fluctuations were found to be attributable to the 2-hop travel time of Whistler mode echoes of the chorus emissions, whereas long (8–16 s) periods were attributed to amplitude modulation by geomagnetic micropulsations.

Next, the chorus was filtered in the narrow frequency band (2–3 kHz), and the filtered output integrated using a time constant of 0.25 s. The integrated waveform was smoothed by means of a d.c. bandpass filter which attenuated statistical fluctuations with periods  $< 1$  s, and removed any ultra-flow frequency trends with periods  $> 100$  s, which are inimical to power spectral analysis, in the integrated waveform. The smoothed waveform was sampled at a rate of  $4 \text{ s}^{-1}$  and digitised to obtain a time series showing the temporal fluctuations in intensity of the chorus.

Figure 3 is a contour map showing, effectively, the evolution of the twelve power spectra computed. The contours were obtained by the following procedure: First the normalised values of the spectral points, especially those defining peaks, in each of the sequential power spectra were denormalised by simply multiplying them by the variance (mean square value) of the corresponding time series used to compute the spectrum. The resultant values were renormalised with respect to the largest value (corresponding to the power in the most dominant period in the entire 60-min recording of chorus) in the set of twelve power spectra, and the values scaled to lie between 0 and

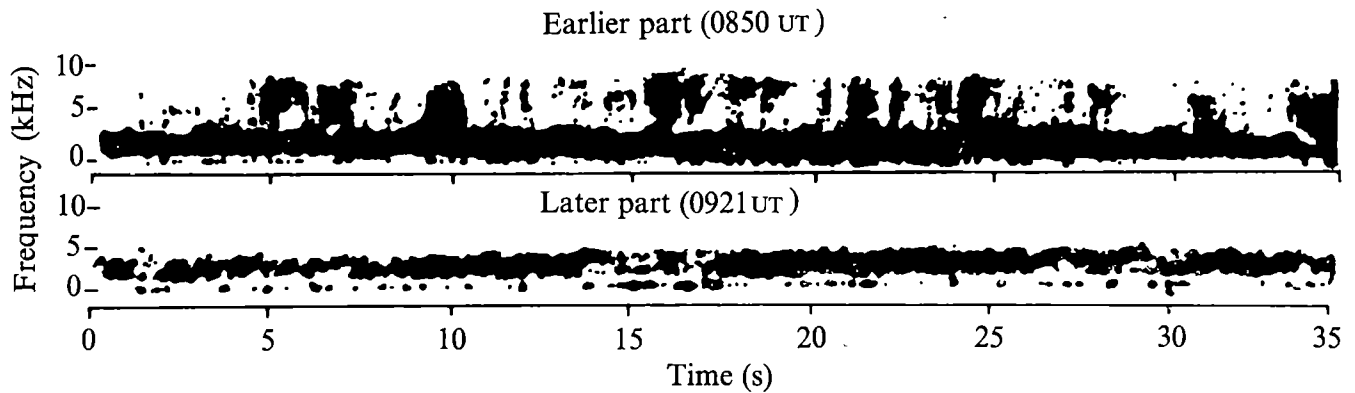


Fig. 1 Dynamic (frequency-time) spectra of samples of earlier and later parts of chorus.

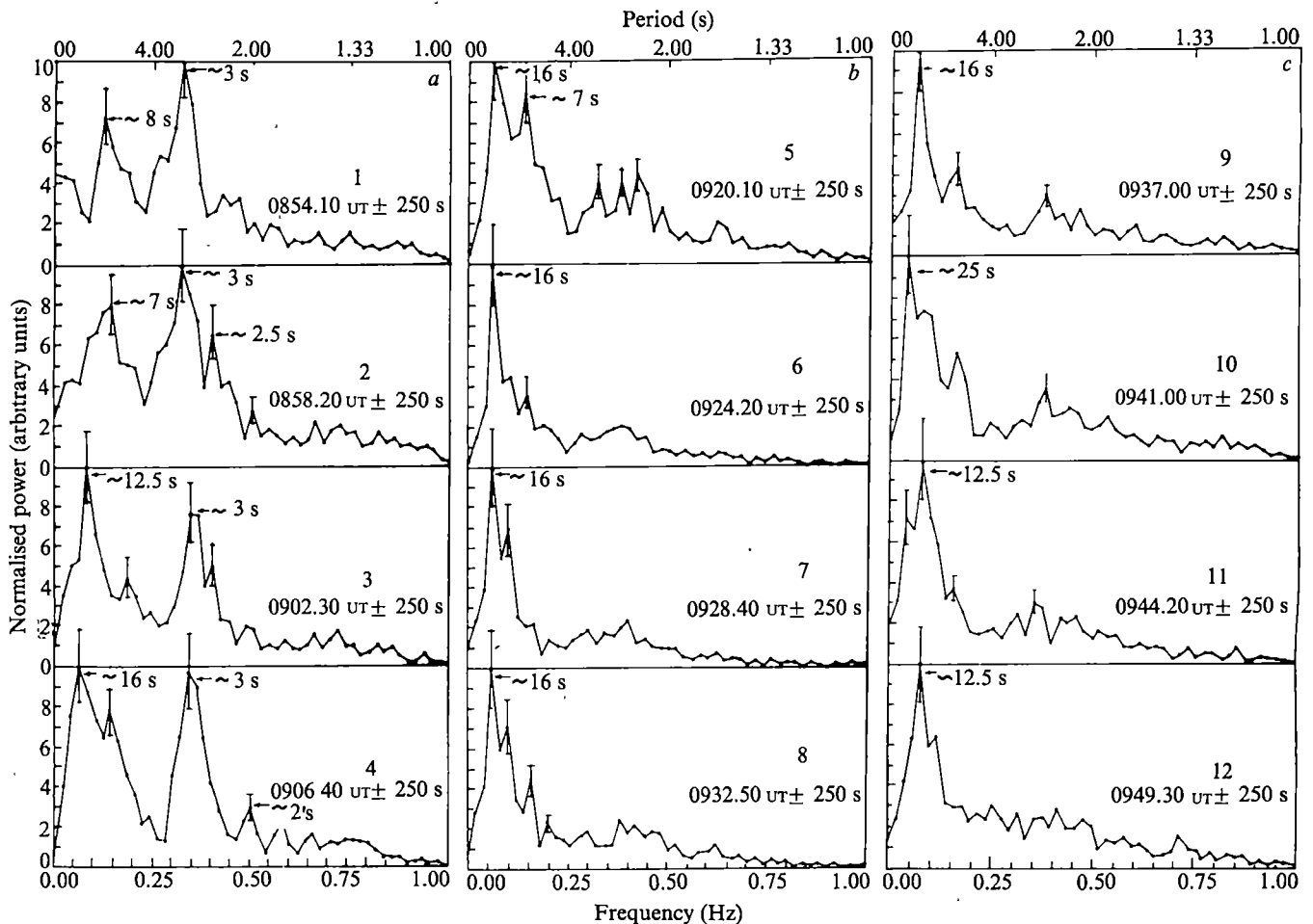
10. These scaled values were plotted for each power spectrum as shown by the dots and crosses in Fig 3, and contours drawn by eye. Significant power peaks or ridges are identified on the contour plot as being high, H, significant valleys are identified as being low, L

It is clear that there is a transition from more complex power spectra for earlier parts of the chorus to less complex ones for later parts. The transition time is  $\sim 25$  min. In the more complex spectra, large spectral peaks defining both short (2–3 s) and comparatively long (8–16 s) periods are present whereas in the less complex spectra, the short period peaks are absent or have small amplitude suggesting that long periods tend to dominate the later parts of chorus.

From a study of chorus recordings from South Uist, Outer Hebrides, Scotland (S.K.A., unpublished), it was

found that short period ( $< 4$  s) fluctuations in the intensity of chorus could be attributed to the 2-hop travel time of whistler-mode echoes of individual (or groups of) chorus emissions whereas comparatively long period ( $> 4$  s) fluctuations are attributable to modulation by geomagnetic micropulsations of the same period. The dominant short period of 3 s observed here is equal to the typical 2-hop travel time of a 2-kHz (the mid-frequency of the chorus examined here) whistler mode echo propagating along the  $L=3.5$  field line, within the plasmopause. The presence of short period fluctuations in intensity of the earlier parts of the chorus suggests that at the peak of the entire chorus event, the emissions have large energy and are able to echo (in the whistler mode) back and forth between conjugate hemispheres perhaps several times before dying off. It also suggests that at the peak of the chorus event some of the

Fig. 2 *a-c*, sequential power spectra of chorus time series



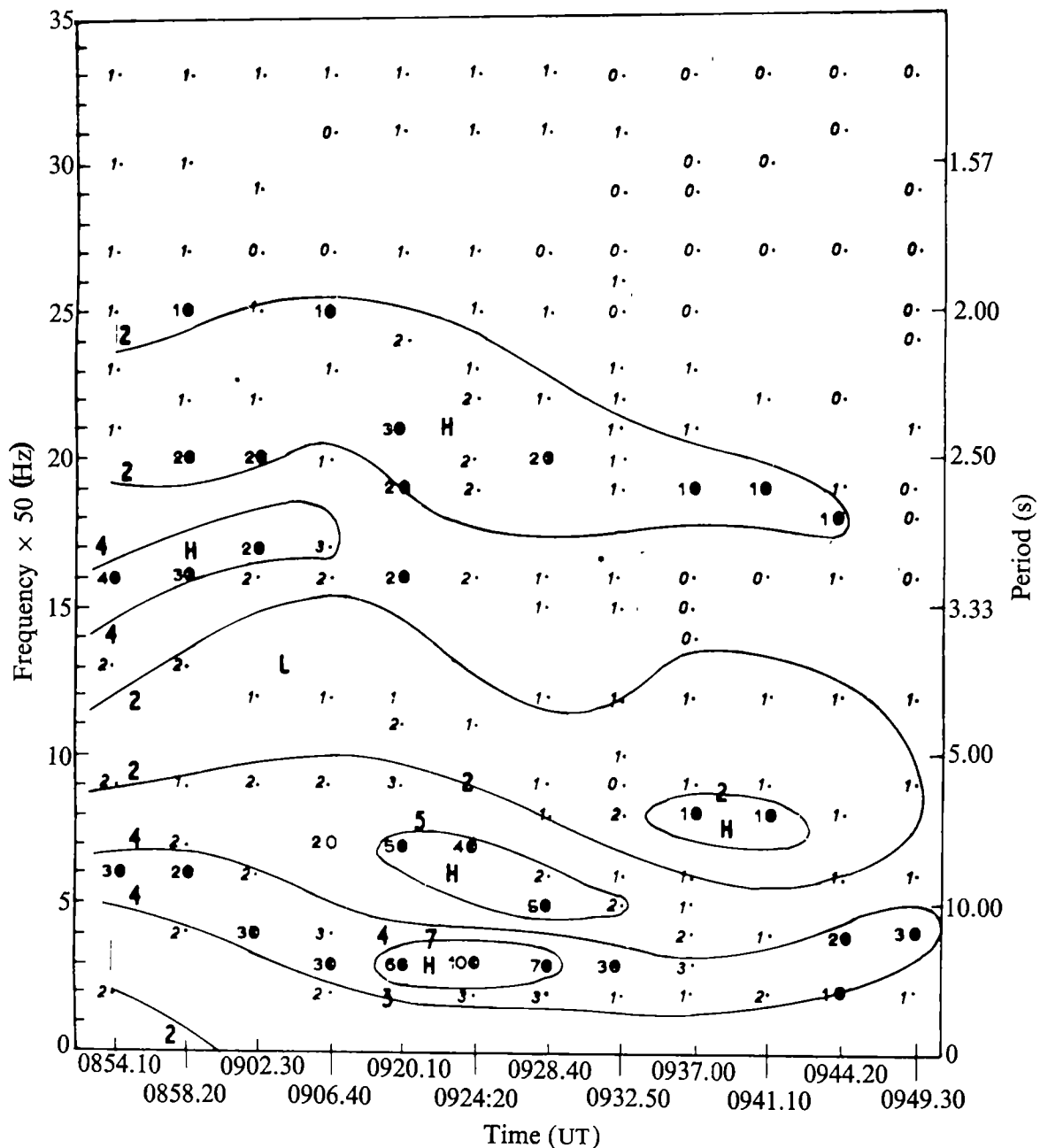


Fig. 3 Contours showing evolution of power spectra.

emissions were being generated inside the plasmopause.

Towards the end of the chorus event, the emissions will have little energy to echo, and so fluctuations in intensity of the chorus will be largely due to amplitude modulation by micropulsations (if present) of the magnetic field line along which the emissions (whistler mode waves) propagate. This kind of modulation, according to Coriniti and Kennel<sup>6</sup> is exponential even for small amplitude whistler mode waves. The dominant long period fluctuation for later parts of the chorus is  $\sim 16$  s; this is in fair agreement with the values of 14 s and 18 s for the eigenperiods of oscillation of the  $L=3.5$  field lines, computed by Orr and Matthew<sup>7</sup>, assuming toroidal and poloidal oscillations respectively, for an electron density of  $15 \text{ cm}^{-3}$ . The low value used for the electron density is typical for a region outside the plasmopause. Thus, it would appear that near the end of the chorus event, the emissions were mostly being generated outside the plasmopause. The presence of short (2–3 s) and comparatively long (8–16 s) periods in power spectra for earlier parts of the chorus time series also supports generation of the emissions in a region close to the plasmopause.

In this case it is possible for the emissions to have been generated or alternatively travelled, some inside, and some outside, the plasmopause.

The evolution of the power spectra of the chorus time-series is, indeed, suggestive of changes in the energy of the plasma medium, available for conversion into chorus emissions. According to the Kennel–Petschek theory<sup>8</sup>, the energy of electrons resonant with whistler mode waves is dependent on the magnetic energy per particle,  $E_e (= B^2/2\mu_0 N)$ . Since the value of the magnetic field  $B$  is dependent on the  $L$  value of the field line, and the value of the electron density  $N$  at the point in the magnetosphere where gyroresonance between the waves and the electrons is taking place depends mainly on whether the point is inside or outside the plasmopause, the evolution observed here may possibly be related to the movement of the position of the plasmopause on a time scale of  $< 1$  h.

The period of micropulsations of any particular geomagnetic field line depends on the plasma density and thus would be affected by changes of the plasmopause position. Thus, one would expect a similar evolution of the power



spectra of geomagnetic micropulsations. It is rather unfortunate that a simultaneous recording of geomagnetic micropulsations was not made at the time of the chorus event to enable a computation of the power spectra of the micropulsation time series to be made and thus to find out whether periods, and an evolution similar to those of the chorus time series would be exhibited. Nevertheless, a similar evolution of power spectra of the time series of micropulsations has been recorded elsewhere (S.K.A., unpublished).

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## Large scale Palaeozoic shear zone in Australia and present extension to the Antarctic Ridge

THE Lower Proterozoic craton of central Australia is bounded to the south and south-east by shallow geosynclinal sediments of late Proterozoic to early Palaeozoic age. During the Palaeozoic these rocks were deformed into a series of upright folds, the trend of which follows the edge of the craton except in the area north and east of Adelaide where it swings to north-north-west-south-south-east, normal to the edge of the craton (Fig. 1). An analysis of this major structure gives an indication of a component of plate movement during the Palaeozoic.

The sigmoidal curve in the trend of the Palaeozoic folds has the form of a ductile shear zone analogous to the small scale, ductile shear structures described from deformed igneous rocks<sup>1</sup>. This is confirmed by the parallelism of isogons drawn for the regional change in axial plane and cleavage orientation (Fig. 2). It suggests that a regional pattern of folds on north-east-south-west axial planes was modified by a sinistral shear couple parallel to the trend of the isogons (Fig. 2).

The simple shear mechanism is also confirmed by the variations in the intensities of deformation. Finite deformation increases as the fold axial planes change in orientation from north-east-south-west to north-north-west-south-south-east. Where the rocks have a north-east-south-west trend, the folding of the cover sequence is largely controlled by the position of structures in the underlying basement which tightened during Palaeozoic deformation. Thus, in the area west of Broken Hill, in the north-eastern sector of South Australia, anticlines in the cover sequence lie over much tighter earlier, antiforms in the basement gneisses. The intensity of deformation in the cover sequence varies locally; conglomerate pebbles at the base of the Upper Proterozoic sequence show the most intense deformation in synclinal keels, where the sediments are pinched into the basement gneisses. In the anticlinal areas, the pebbles show no apparent change of shape, and the sediments may show no cleavage. East of Adelaide, where the folds have a north-north-west-south-south-east trend, folding is more regular and better developed, conglomerate pebbles show

more intense deformation throughout the fold structures, and most of the rocks have a well developed cleavage. Deformation is heterogeneous, presumably as a result of the simple shear acting on an originally heterogeneous deformation pattern.

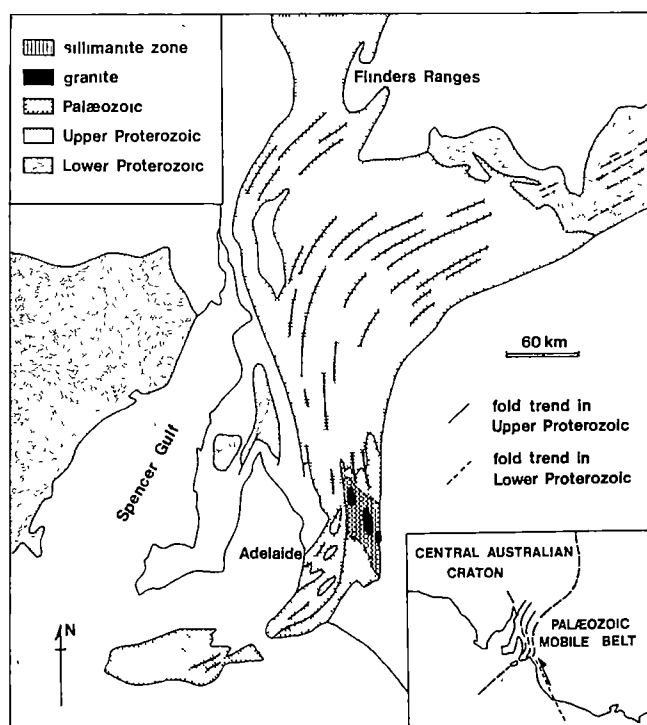
The intensity of deformation attributable to the simple shear component can be estimated from the change in orientation of the fold axial planes or cleavage, relative to the shear direction<sup>1</sup>. Thus, the shear strain can be computed throughout the zone, with a maximum shear strain,  $\gamma$ , of 1.7 (a strain ratio of 4.5:1) some 30 km north-east of Adelaide (Fig. 2).

The zone of most intense shear strain coincides with a zone of sillimanite-grade metamorphism and migmatization (Fig. 1). The high heat flow required to give this local increase in metamorphic grade may result partly from intense shear strain; or alternatively, the position of the shear zone may have been controlled by the increase in ductility over a region of high heat flow. Evidence for that hypothesis has been given by Fleming and Offler<sup>2</sup>; they showed that the high grade metamorphism began before the development of the first cleavage, and continued through two later phases of localised deformation of crenulation cleavage.

The ductile shear zone is 150 km wide, and shows left-lateral displacement of the order of 150 km (Fig. 1). In terms of plate tectonics, the zone cuts across the edge of the Australian craton and must be intracontinental; the eastern part of the Australian plate must have moved northwards some 150 km relative to the western part. Seismic evidence indicates that there may be a gradual change in crustal structure under the northern extension of this shear zone<sup>3</sup>, and studies of electrical conductivity have detected an anomaly under the Flinders Ranges in the northern part of the zone. This may reflect a change in rock fabric at depth<sup>4</sup>.

The ductile deformation along the shear must have ceased during the Palaeozoic; it was presumably most active during the mid-Palaeozoic at the time of high grade

Fig. 1 Geological map of part of South Australia showing the sigmoidal trend of the fold axial planes and the area of high grade metamorphism.



metamorphism and granite production. Large faults, however with a similar north-west-south-east trend to the shear, postdate the deposition of Permian sediments.

From examinations deep crustal seismic profiles, Burwash *et al.*<sup>5</sup> suggested that major faults and shear zones in the Churchill Province of Canada extend down to the mantle, and Beach<sup>6</sup> claimed that the major shear in the Laxford region of north-western Scotland almost certainly penetrated to the mantle. According to Beach<sup>6</sup>, shear zones in north-western Scotland extend for a distance of the order of 10 times the amount of displacement. By analogy, the South Australian shear zone, with a displacement of 150 km can be expected to extend for some 1,500 km. Shear zones of such size most certainly affect the base of the lithosphere, and the change in rock fabric in the upper mantle<sup>4</sup> beneath the Flinders Ranges presumably represents such deformed asthenosphere.

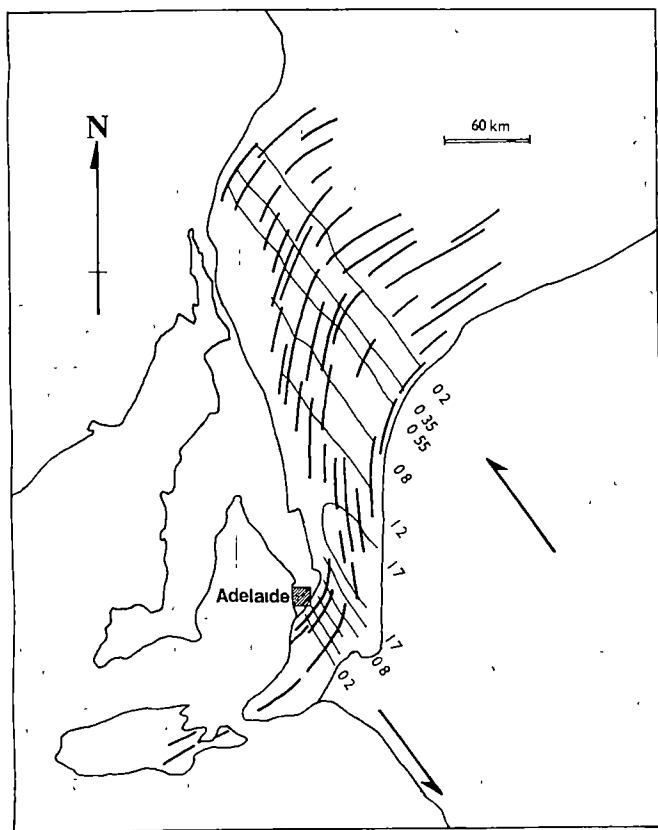


Fig. 2 Isogons drawn for the regional change in the orientation of fold axial planes. Estimated shear strains are shown.

Watterson<sup>7</sup> has pointed out that the development of fabric and grain size reduction within shear zones produces a localised strain-softening effect so that major shear zones may thus be active persistently throughout geological time. Doyle *et al.*<sup>8</sup> have shown that the Adelaide region of South Australia is a zone of recent seismic activity. Cleary and Simpson<sup>9</sup> have extended this zone of seismicity to a seismic zone and a major transform fault on the Antarctic Ridge. They consider that the whole zone from South Australia to the Antarctic Ridge represents a zone of left-lateral differential shear movement formed by differences in spreading rates between different parts of the Australian plate. This is in accord with the suggestion of Wilson<sup>10</sup> that transform faults on mid-oceanic ridges originate on lines of weakness within continents. Thus, the transform fault on the Antarctic Ridge was formed where the vertical zone of sheared lithosphere and asthenosphere was crossed

by a spreading ridge along which there was a gradient in the spreading rate.

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## Magnetic inclination of basaltic lavas from the Mid-Atlantic Ridge near 37°N

DURING the course of the French-American Mid-Oceanic Undersea Study (FAMOUS), many samples of basaltic pillow lava were collected from the axial region of the Mid-Atlantic Ridge near 37°N. The vertical axes and the tops of nine of these samples have been determined fairly accurately, allowing calculations of magnetic inclination at the time of the cooling of the lavas to be made with reasonable confidence.

According to modern conceptions of seafloor creation, basalts from the axial regions of the mid-ocean ridges should be young, and therefore magnetised in approximately the same direction as the present geomagnetic field. But the few magnetic measurements that have been made on basalts collected irrefutably on the crustal block responsible for the existence of the axial magnetic anomaly have yielded conflicting results<sup>1-3</sup>. Normal polarity has been found in samples from the Reykjanès Ridge<sup>1</sup> and from the Mid-Atlantic Ridge near 45°N (refs 2-4), but mixed or reversed polarities were found in two of six basalt cores drilled within the Rift Valley at the same latitude<sup>5</sup>. The mean magnetic inclination at a site cannot be reliably calculated if the number of satisfactorily reoriented samples is too small or if the direction of magnetisation is erratic within a single pillow or between samples. Although these conditions seem to have been met for samples dredged from 45°N (ref. 4), the mean magnetic inclination is 20° lower than expected.

Three of the samples discussed here (CH 31-DR 1-111; CH 31-DR 2-122; CH 31-DR 4-200) represent material dredged during the RV Jean Charcot CH 31 cruise of 1972 (refs 6 and 7). The remaining six samples (ARP 73-10-02; ARP 74-10-15; ARP 74-11-17; ARP 74-13-24; ARP 74-14-31; and ARP 74-17-40) were collected by a telemanipulated arm operated from the bathyscaphe Archimède during the FAMOUS diving programmes of 1973 and 1974 (refs 8 and 9). The locations of the sampling sites are shown in Fig. 1. Six samples were collected in the axial part of the Rift Valley, the remaining three come from the region of the intersection of the Rift Valley and Transform Fault 'A' near 36°57'N. The age of the samples can be inferred from a variety of methods.

The oxygen isotope ratio for a rock from dredge haul CH 31-DR 1 indicates that the material is very fresh, having suffered no noticeable seawater contamination (G. Pineau, M. Javoy and J. Bottinga, personal communication). Samples from dredge CH 31-DR 2, dated on a relative scale by measurements of the thickness of the palagonite-manganese coating<sup>10</sup> are dated at less than 170,000 yr BP. No age determination is available yet for samples from dredge CH 31-DR 4.

The youngest rock of the set of Archimède samples in the inner floor of the Rift Valley is probably ARP 74-10-15,

**Table 1** Magnetic properties of specimens of pillow lavas from the rift valley of the Mid-Atlantic Ridge near 37°N

Location*	Sample number	Specimen	$J_0^\dagger$	MDF	$I_{s0}^\S$	$I_s^\S$	$\theta^\P$	$I_c^{  }$	$I_c^{**}$
1	CH 31-DR1-111	U (G)	23.4	834	68.7	68.3	2.2	67.2	67.2
		L	220	325	63.8	66.1			
2	CH 31-DR2-122	U	173	243	66.1	65.4	1.5	64.7	64.7
		L (G)	64.5	311	64.2	63.9			
3	CH 31-DR4-200	U (G)	16.9	130	53.2	50.4	3.7	50.0	50.0
		L	109	554	35.2	49.6			
4	ARP 73-10-02 (1)	U	405	181	45.1	43.3	8.9		
		L	170	122	41.3	47.9			
	ARP 73-10-02 (2)	U	288	113	54.7	53.9	3.7	53.7	53.7
		L	171	153	52.5	53.5			
5	ARP 74-10-15 (1)	U (G)	108	495	56.7	55.7	2.3	56.5	56.5
		L	503	226	51.7	57.3			
	ARP 74-10-15 (2)	U	246	300	48.6	55.8	11.2		
		L	455	226	59.9	60.2			
6	ARP 74-11-17 (1)	U (G)	43.5	877	70.1	70.4	0.9	70.7	
		L	348	141	71.4	70.9			
	ARP 74-11-17 (2)	U (G)	33.5	806	69.6	69.2	0.7	69.5	70.5
		L	319	156	69.1	69.7			
	ARP 74-11-17 (3)	U (G)	56.8	849	69.9	70.2	2.5	71.2	
		L	262	170	68.7	72.3			
7	ARP 74-13-24 (1)	U	99.0	444	44.7	43.4	13.8		
		L	67.2	229	23.9	30.7			
	ARP 74-13-24 (2)	U	92.2	474	43.1	46.2	13.5		
		L	62.0	141	29.0	36.7			
	ARP 74-13-24 (3)	U	107	433	44.1	45.1	9.2		
		L	65.8	181	24.3	36.6			
8	ARP 74-14-31 (1)	U (G)	17.7	693	47.4	47.4		47.3	
		L	54.1	523	44.3	47.1			
	ARP 74-14-31 (2)	U (G)	28.3	543	50.9	50.7	4.6	48.7	48.0
		L	78.1	331	42.5	46.7			
	ARP 74-14-31 (3)	U (G)	33.9	639	47.6	49.1	7.4		
		L	49.5	475	49.3	48.2			
9	ARP 74-17-40 (1)	U	106	83	42.1	42.1	1.8	41.7	
		L	99.8	192	25.1	41.2			40.7
	ARP 74-17-40 (2)	U	117	85	58.5	40.0	2.2	39.7	
		L	153	113	41.3	39.3			

\* Location refers to numbered sites in Fig. 1. Sample number is eventually followed by core number (in brackets); U, upper specimen; L, lower specimen; G, glass on specimen.

$\dagger J_0$ , mean intensity of magnetisation before cleaning ( $10^{-4}$  e.m.u.  $\text{cm}^{-3}$ )

$\ddagger$  MDF, median destructive field in peak oersted.

$\S I_{s0}$  and  $I_s$  are magnetic inclinations before and after cleaning, respectively.

$\P \theta$ , angular deviation between the directions of magnetisations of the two specimens from the same core.

$|| I_c$ , mean inclination of core

\*\*  $I_p$  mean inclination of pillow.  $I_s$ ,  $\theta$ ,  $I_c$  and  $I_p$  calculated after a.f. demagnetisation.

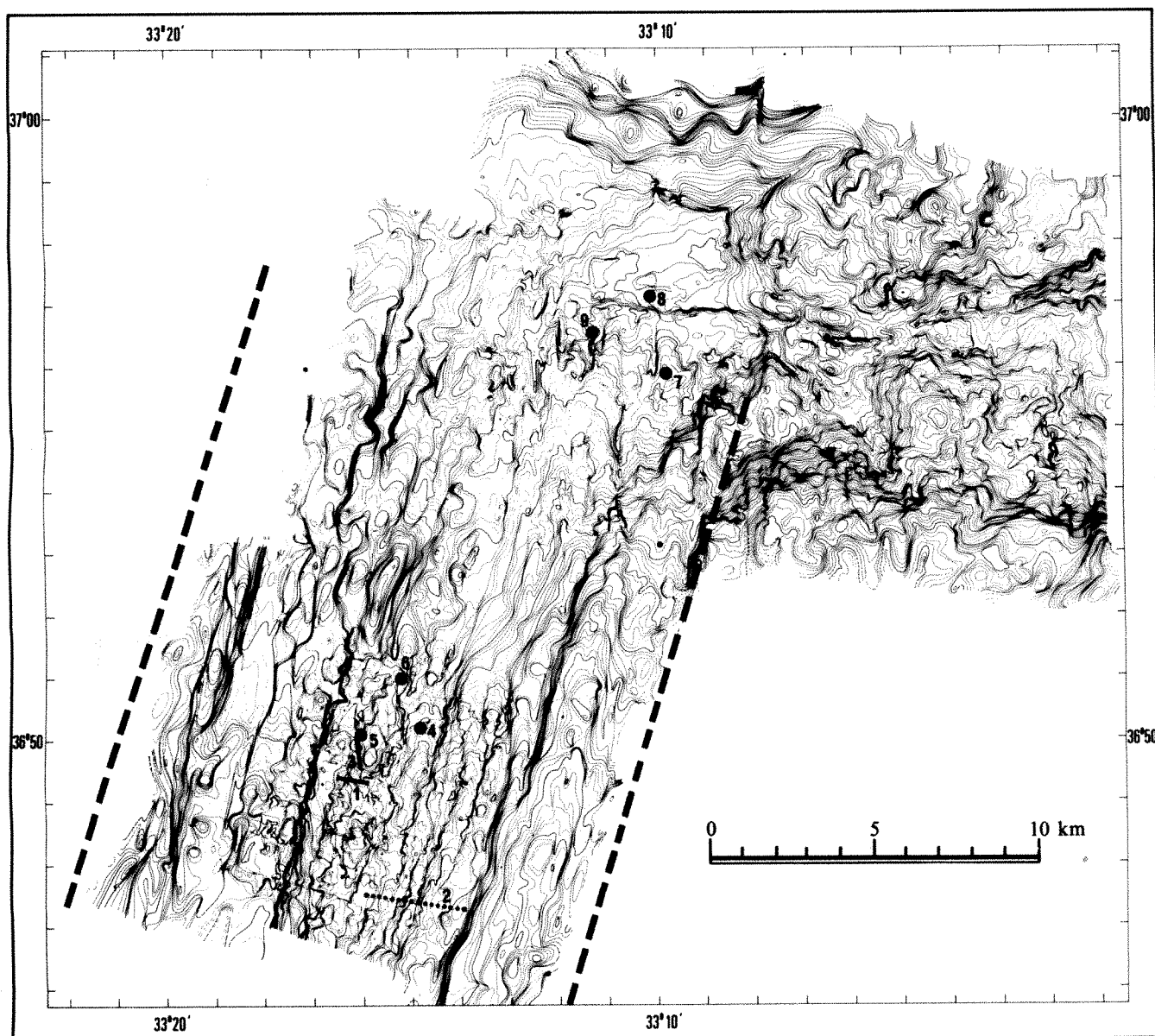
which exhibited fresh glass on the chilled margin, followed by ARP 74-11-17, which was sampled in a talus pile barren of sediment, and which showed very little preserved glass. Both are olivine basalts<sup>7,9</sup> representing the youngest eruptives in that section of the Rift Valley, probably emplaced within the past 10,000 yr (refs 10 and 11). The age of sample ARP 73-10-2 is bracketed between 30,000 and 100,000 yr on the basis of these palagonite-manganese coatings<sup>10</sup>. Fission-track dating (D. Storzer, personal communication) shows that this sample was formed less than 50,000 yr ago.

The remaining three Archimède samples (ARP 74-13-24; ARP 74-14-31; and ARP 74-17-40) come from the axial portion of Transform Fault 'A' in the region close to its intersection with the Rift Valley. Sample ARP 74-13-24 was taken 4 km east of the axis of the Rift Valley segment on a small north-south ridge which structurally is part of the Rift Valley wall. Its age should be about 270,000–300,000 yr according to spreading rates for the eastern limb of the Rift Valley, calculated from magnetic profiles<sup>8,12</sup>. Sample ARP 74-14-31, with a rosey surface showing flow direction, has a preserved glassy crust and a palagonite layer overlain by a thin manganese coating. Samples at this site, on the edge of a tension gash associated with the left lateral shear of Transform Fault 'A' (ref. 9), are the youngest specimens collected in the Transform Fault. This estimate is supported by a fission-track age (D. Storzer, personal communication) of less than 45,000 yr. Sample ARP 74-17-40, closest to the accreting plate boundary, has textural and structural features similar to those of rocks determined to be intru-

sives from field evidence. It was sampled on a tectonic scarp which exhibits a layered sequence (possibly a truncated sill) 1.5 km east of the deep axis of the Rift Valley. Its age, inferred from spreading rates, should be less than 100,000 to 113,000 yr (refs 6 and 12). Spreading rate values averaged over the Brunhes Epoch may not necessarily, however, be applicable to an area which may be tectonically and volcanically active. Fission-track ages for this general region are all less than 300,000 yr (D. Storzer, personal communication).

On the combined evidence of fission-track dating, calculated opening rates, palagonite and manganese thickness, and field observations, it seems certain that all the reoriented rocks were emplaced during the Brunhes Epoch, so that a proper scheme of reorientation should reveal that the samples are all normally magnetised. Any negative inclinations could be attributable to eruptives that cooled during the Laschamps (reverse) geomagnetic excursion, which may have occurred between 10,000 and 15,000 yr BP, or during the Blake reverse event (or excursion) with an inferred duration of about 10,000 yr and a mean age close to 110,000 yr BP (see ref. 5).

The partial reorientation of samples from the sea floor is possible first, for cores, assuming that the axis of drilling is vertical (in practice, deviation from the vertical can reach 15°; ref. 5 and, second, for magnetically viscous samples, using various magnetic tests; refs 2, 13 and 14), (these methods should obviously be applied only to rocks whose viscosity has been established; they cannot be used for the samples discussed here because their viscosity index



**Fig. 1** Bathymetry of parts of the Rift Valley and Transform Fault "A" (21) showing sampling sites (Table 1) and boundaries (heavy dashed lines) of the crust formed during the Brunhes Epoch<sup>6</sup>. The sampling sites are taken from refs 6, 8 and 9. The location of dredge 2 (dotted line) is only indicative. The numbered locations refer to the first entry in Table 1. The Rift Valley sites are all clearly within the Brunhes block and should therefore have normal polarity.

is too small<sup>15</sup>); third, for pillow-lavas exhibiting one or more volcanic features considered to be criteria of polarity<sup>1,4</sup>.

The last method is the only one which gives indications of the orientation of the pillow-lava during cooling because it is independent of possible subsequent changes of position.

De Boer *et al.*<sup>1</sup> have listed four volcanic features thought to be diagnostic of vertical and top: the flattened-oval shape of pillows; the location of necks interconnecting pillows at their bases; stemlike extensions commonly at the bases of pillows; and basal stalactites on the concave side of a crust from lava tunnels or tumuli. Of these, the last seems to be the most reliable (see, for example, ref. 16) but it can give only a polarity and cannot give the vertical because the position of the lava crust with stalactites on the tumulus or tunnel cannot be known *a priori*. In another study<sup>4</sup>, the horizontal was assumed to be parallel to the equatorial plane of the pillow but the assumed error of 20° (ref. 4) is probably too low, because the method relies strongly on questionable conceptions of how pillows form<sup>16</sup>, and assumes that the pillow cooled on a horizontal surface. A further possibility, that of using surface glass coating as

a guide to the original orientation, is not reliable because pillows often curl during their formation.

An improved criterion of orientation came from field observations made from the American submersible Alvin in the inner floor of the Rift Valley in the FAMOUS area<sup>17-19</sup>. It was noted that, on the tops of lava flows, series of ledges commonly occur along the inner walls of collapsed feeder tubes. The ledges record the various levels at which lava flowed through the tubes<sup>17</sup> much as rings in a bathtub register successive water levels<sup>18</sup>. These frozen-in lava levels were observed to be nearly horizontal *in situ*, and they provide a reliable guide to sample orientation for palaeomagnetic studies<sup>19</sup>. The nine samples for which measurements are reported here are the only ones among all the rocks sampled by the French team in the FAMOUS area showing the 'bathtub ring' criterion or a modification of the criterion. Some examples are shown in Figs 2 and 3.

In sample CH 31-DR 4-200 (Fig. 2b), a small flattish ledge occurs near the core of a pillow exhibiting a glassy margin. The upper surface of the ledge is very smooth and contrasts with the overlying, subparallel wall, which has a



rugose texture imposed by the presence of small septae and lava stalactites. The vertical is taken normal to the flattish ledge and the polarity is given by the relationship between the rugose stalactite-bearing surface and the smoother, upward-facing surface. The flattish glassy surface is tilted about  $30^\circ$  from the horizontal.

The angular discrepancy between the glassy surface and the horizontal is more extreme in the case of sample ARP 73-10-02 where the glassy surface is nearly vertical (Fig. 2c). In the interior of the pillow a series of elongate cavities with smooth flattish floors and more irregular roofs gives a good indication of the vertical at the time of cooling.

In sample ARP 74-11-17 (Fig. 2d), the glassy surface is on top and nearly horizontal. The vertical is taken to be normal to a line of small, elongate, asymmetric cavities. The true vertical could deviate slightly, because the cavities seem to be *en échelon* (Fig. 3) and the vertical axis could have been taken perpendicular to the parallel long axes of the cavities. The polarity is not, however, in doubt and is given by both the asymmetry of the cavities and by the lava stalactites on a large surface (Fig. 3).

Sample ARP 74-14-31 shows a superposition of two, approximately horizontal, ropey lava surfaces (Fig. 2a). A confirmation of the field determination is provided by an elongate cavity below the upper ropey crust and by well developed stalactites on the bottom of the sample.

Suitable samples for reorientation could not be found in the collection made from the diving saucer Cyana, because of their small size. Similarly, only the very large dredge samples could be used with any confidence.

One to three vertical cores, about 10 cm long, were drilled through each of the nine samples of reoriented pillow lava basalts. The upper and the lower parts of each core were sawn flat, giving two specimens of about 30 g. All 36 specimens were submitted to magnetic study, including alternating field treatment up to 850 oersted (Table 1). In several cases, the intensities of magnetisation and the median destructive field (MDF) were quite different from one specimen to another, even within a single core. In particular, the glassy parts of a sample are systematically much less magnetised than non-glassy parts. The very high coercivities shown by

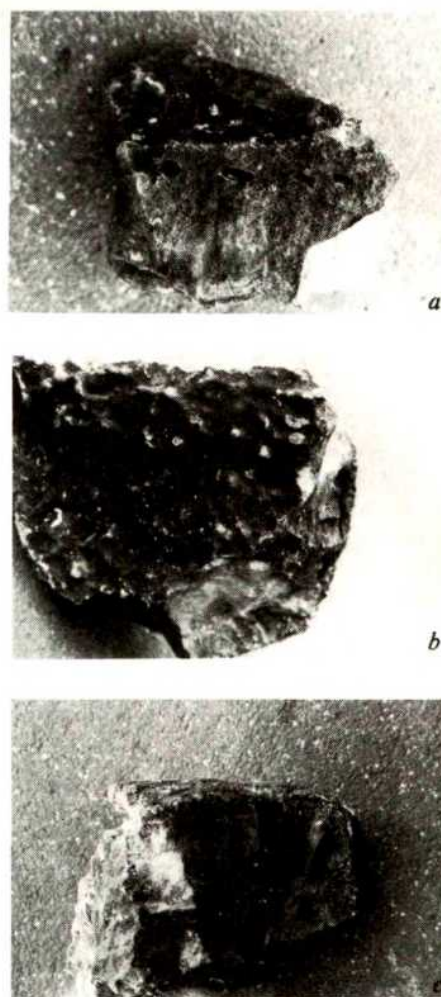
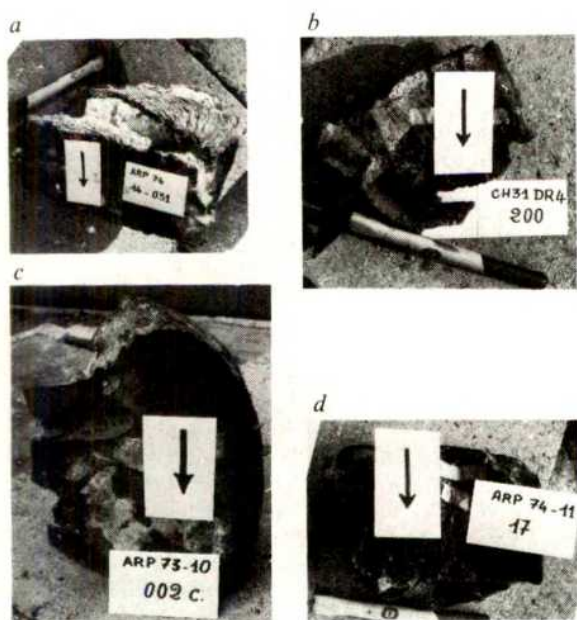


Fig. 3 a, Close-up of cavity alignment in sample ARP 74-11-17 (Fig. 2 d); b, stalactite-bearing surface (vertical-upward into picture) at the base of ropey lava ARP 74-14-031 (shown in Fig. 2); c, close-up of horizontal cavity below upper ropey surface of sample ARP 74-14-031 (Fig. 2a).

Fig. 2 Four selected pillow lavas displaying characteristic features for reorientation (see text). Arrows, vertical-downward at the time of cooling. a, ARP 74-14-031; b, CH 31-DR-4-200; c, ARP 73-10-02C; d, ARP 74-11-17.



the glassy portions of the samples probably reflect in part the smaller grain size. As previously pointed out<sup>22</sup> the between-specimen variability indicates that large samples (or the averaging of results from several specimens from each pillow) are generally required to obtain significant data on the intensities of magnetisation of submarine extrusives.

All of the specimens have a positive magnetic inclination. The alternating field treatment induced small changes in direction, probably by the removal of weak parasitic magnetisations acquired during drilling. Because the samples are not viscous, no significance can be attached to the sign of the inclination change during cleaning. In some cases, the directions of magnetisation after cleaning remain significantly different for the upper and the lower specimen from a single core. In particular, the upper part of pillow ARP 74-13-24 is characterised by a magnetic inclination  $10\text{--}15^\circ$  higher than that of the lower part of the pillow. This may indicate variations in space of the geomagnetic field at the site of cooling, because of disturbances caused by the neighbouring lavas. The large inclination found for sample ARP 74-11-17 may be real and result from secular variation. Alternatively, it may be the result of a small error in the orientation, as discussed above.

In calculating the mean inclination of a pillow we have rejected data obtained from cores exhibiting a deviation larger than  $5^\circ$  in their directions of magnetisation (after cleaning) between the upper and the lower part of the pillow.

The overall mean magnetic inclination, calculated from the mean values for eight pillow basalts, is equal to  $56^\circ$ . It corresponds exactly with the inclination of the present geomagnetic field (International Geomagnetic Reference Field, 1965), which is the same as the inclination of an axial dipole field. At two drill sites of DSDP leg 37, west of the FAMOUS area, natural remanent inclinations were also found to be close to the dipole value for the area and to match this local anomaly sense<sup>20</sup> although anomalous shallow inclinations were found at a third site.

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## Dune trend and the Ekman Spiral

FRICTION at the Earth's surface induces deflection of the ground wind from the geostrophic direction, the progressive change with height being known as the 'Ekman Spiral'. As the roughness of the surface increases, this deflection becomes further to the left in the Northern Hemisphere and further right in the Southern; the differences between extensive water or grass-covered areas and nearby rough surfaces, such as rooftops, are commonly  $15^\circ$  (ref. 1). The evidence presented here shows that dune fields, many of which have rougher surfaces than neighbouring areas, and within which there may be differences in roughness between dune types, show evidence of such deflections.

Deflection is most apparent in coastal dunes, probably because wind recording stations are relatively common on coasts, and because the important onshore winds encounter a marked contrast between the surface roughness of the sea and that of the dunes.

Landsberg<sup>2</sup> examined twelve parabolic dune fields on the coasts of Britain and Denmark; in eight of these the dune trends are to the left of the resultant of sand movement at the nearest wind recording station (that is, the average wind direction weighted by its ability to blow sand), and in two there is little difference. The site at Burghead in Scotland

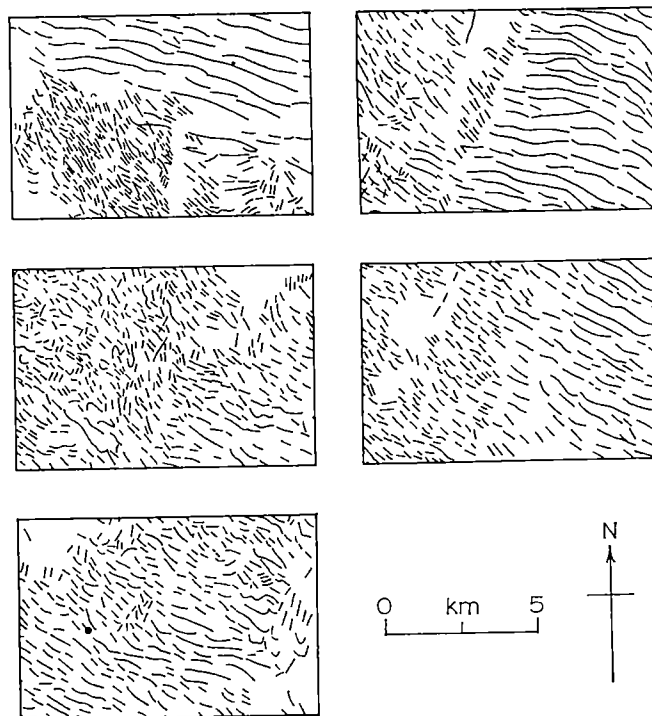


Fig. 1 Patterns of transverse-type dunes in the fixed Qoz sand dunes of Central Sudan. Each line represents a dune crest; the winds came from a northerly direction. The wind at right angles to the longer wavelength dunes is always to the left of that normal to those with lower wavelengths

shows too marked a leftward deflection to fit with Landsberg's explanation that it is the result of the occlusion of landward winds at the recording station. The sites which show a rightward deflection do so when compared with very distant recording stations. In Gower, South Wales, Harris<sup>3</sup>, like Landsberg, invoked sheltering to explain the discrepancy between the trend of the sand moving resultant and the dunes, but here too there is a distinct leftward deflection of the dunes.

In the dune fields of the Oregon coast there is a consistent difference between the trend of the dominant winds and of the dunes: lines perpendicular to nearshore transverse dunes have an orientation from  $1.5^\circ$  to  $22^\circ$  to the left of the dominant NNW winds which are thought to form them<sup>4</sup>. One recording station, North Bend, which is some km inland from dune country, shows that the dominant wind has swung to the left (it is more westward than at the coastal stations). Cooper's<sup>5</sup> data for the California coast are sparser than those for Oregon, but again nearshore dunes set consistently to the left of the July north-westerlies which are apparently responsible for them.

In the Southern Hemisphere the trend of parabolic U-dunes on the eastern, western and southern coasts of King Island, Tasmania is almost everywhere to the right of the sand moving resultant of the onshore winds<sup>6</sup>.

Turning now to the internal evidence of the dune patterns themselves, it can be seen on both Landsberg's<sup>2</sup> and Jennings's<sup>6</sup> maps of parabolic U-dunes that the deflections consistently become more pronounced inland, presumably in response to increased surface roughness. In Oregon the direction at right angles to the mean trend of low transverse dunes is skewed well to the left of the trend of more longitudinal near-shore lee features, which presumably have lower surface roughness<sup>4</sup>. Further inland, the leftward swing becomes pronounced in the long seif-like dune ridges. Cooper related the trend of these to the yearly resultant of sand moving winds (although he did not weight these for sand movement), but the wind records at North Bend, mentioned above, support the idea that the dominant



winds veer to parallel the ridges inland. On the California coast, all the transverse ridges of Flandrian age shown on Cooper's maps<sup>5</sup> imply winds to the left of the trend of the nearshore longitudinal lee features. On the coast of Israel, transverse dunes again indicate a wind to the left of closely associated longitudinal features (a fact which Rim explained as due to an asymmetry in the sand supply)<sup>7</sup>

Desert dunes are not as likely to show such clear evidence of discordance as coastal dunes because wind recording stations are very sparse and are themselves often in terrain as rough or rougher than dune surfaces, and also wind regimes are more complex, since they include relevant winds from many more directions than the simple onshore winds responsible for most coastal dune forms. In spite of these difficulties, many inconsistencies of trend can be explained with the Ekman Spiral. It is found that, as with coastal dunes, the rougher-surfaced transverse dunes are commonly skewed more than longitudinal dunes.

Sprigg<sup>8</sup> suggested that there had been a 4°–5° southward shift in windbelts since the time of dune formation in central Australia, because the sand ridges trend to the right of the wind, but a simple application of the Ekman Spiral shows that if the wind recording stations were in smoother terrain, the dunes should indeed show this trend. The common rightward asymmetry (though not the orientation) of these ridges has been explained with the Spiral by Mabbutt and others<sup>9</sup>. Brookfield<sup>10</sup> found no simple pattern of deviations, but did note that well developed ridges in the Simpson Desert were aligned to the right of the resultant sand movement, and more recently Twidale's<sup>11</sup> maps shows that, in most cases, transverse-type dunes in the same desert would be perpendicular to winds which are to the right of those which parallel the sand ridges.

In the Northern Hemisphere, Clos Arceduc<sup>12</sup> has repeatedly used air photographs to show that long seif or 'slouk' dunes cannot be longitudinal to the winds flowing over nearby barchans. Most of his seifs are to the right of this putative wind, a fact which could be explained if the wind deviated to the left of the geostrophic direction as it passes over the rougher surface of the transverse barchans. Indeed over groups of barchans the deviation is more marked than over single ones (compare numbers 31 and 34 with 37 on Fig. 2 of ref. 12). In the Ténéré Desert, Warren<sup>13</sup> showed that the wind at right angles to transverse zibar would be 14° to the left of one parallel to closely associated seif dunes, and nearby, near the oasis of Bilma, a small dune field of seifs and barchans again indicates that the wind at right angles to the barchans would be to the left of the main trend<sup>14</sup>.

Hack's<sup>15</sup> maps of the Navajo county of the south-western USA show yet again that transverse dunes would be perpendicular to winds which are to the left of long straight seif-like dunes. Near the Salton Sea, Long and Sharp<sup>16</sup>, using wind records from an admittedly distant station, noted that barchans had moved to the left of the dominant winds, during both a 7-yr and a 15-yr period.

The evidence of desert dunes is not, however, entirely in favour of this Ekman Spiral hypothesis. In a pattern in Libya which was very similar to the one in the Ténéré discussed above, McKee and Tibbetts<sup>17</sup>, found that transverse zibar indicated a wind to the right of the one which would parallel the local seifs, and further south in southern Niger, Grove and Warren<sup>18</sup>, found megadune patterns east of Termit and south-west of Agadem in which the transverse features are again apparently aligned to a wind to the right of the one parallel to the longitudinal ones.

The morphology of Pleistocene-fixed dune sands is difficult to interpret in the absence of wind data for the period of their formation, but there is some evidence of divergences over different dune types. In Central Sudan<sup>19</sup> transverse dunes with long wavelengths (rougher surfaces) are invariably at right angles to winds which would have been to

the left of those perpendicular to juxtaposed transverse dunes of lower wavelengths (less rough) (Fig. 1). These large areas of uniform roughness may offer an opportunity to measure the value of surface roughness parameter.

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## Electrical properties of magnetisable liquids

RECENTLY developed magnetisable liquids comprising colloidal suspensions of soft ferromagnetic particles have attracted considerable scientific and technological interest<sup>1–3</sup>. The ferromagnetic grains are coated with a special film which prevents them from clustering together and maintains a true colloidal dispersion<sup>4</sup>. We have investigated the sensitivity of their electrical properties to magnetic fields and found some new 'magneto-electric' effects. An interpretation of these observations is suggested.

Conductance and capacitance measurements of a commercial magnetisable liquid (Ferrofluid water base, A01, 200 gauss saturation, Ferrofluidics Corporation) were made using a Metrohm conductivity probe (type E A 608). This is of a standard type for conductivity measurements and consists of a pair of parallel electrode plates coated with porous platinum. The conductivity of the fluid between the plates  $\sigma$  is obtained from the measured conductance  $S$  through the geometrical factor  $c$ , where  $c \approx \sigma/S \text{ cm}^{-1}$ .

For the probe we used the value of  $c$  was stated by the manufacturers to be  $c = 0.83 \text{ cm}^{-1}$ . Capacitance measurements were made with the same probe. All the a.c. measurements were made on a General Radio type 1650-B bridge operated with an external signal generator.

The frequency dependence of conductivity and capacitance for the liquid is shown in Fig. 1. Note that, although the conductivity is approximately constant over the frequency range, the capacitance varies as  $f^{-2}$  over most of the range. At low frequencies the measured capacitance becomes very large ( $\approx 1 \mu\text{F}$ ).

Figure 2 represents the d.c. characteristic of the magnetisable liquid. As in the case of the a.c. measurements, the geometrical factor  $c$  may be used in order to derive the conductivity from the measurements. Below 5 V, the voltage corresponding to a steady current takes some time to reach a steady value, indicating that not only is the resistance larger than the measured a.c. values, but the capacitance is also significantly larger than those found with a.c. down to 40 Hz. The time constant of 0.5 V was  $\sim 3 \text{ min}$ .

Similar experiments were performed in the presence of magnetic fields, to determine the effect of applied homogeneous and inhomogeneous fields on the electrical properties of the liquid. A solenoid coil wound round the entire column of liquid was

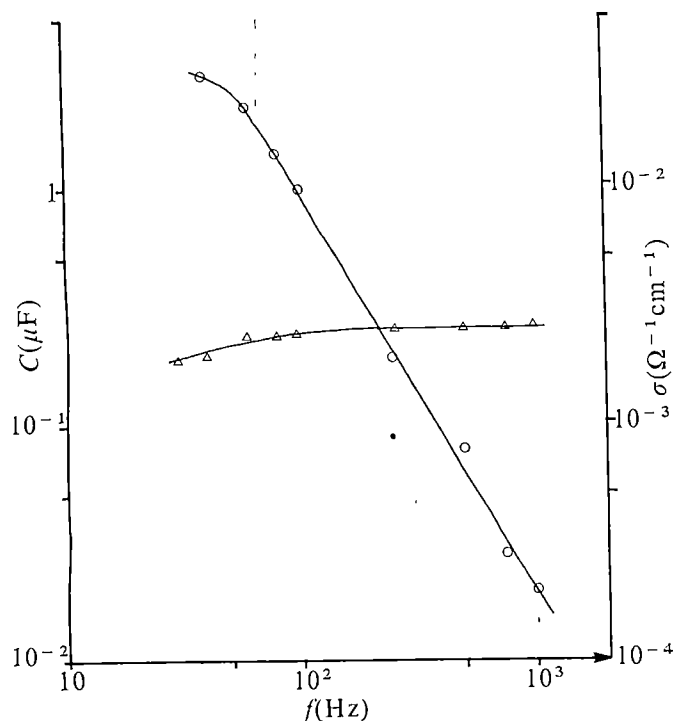
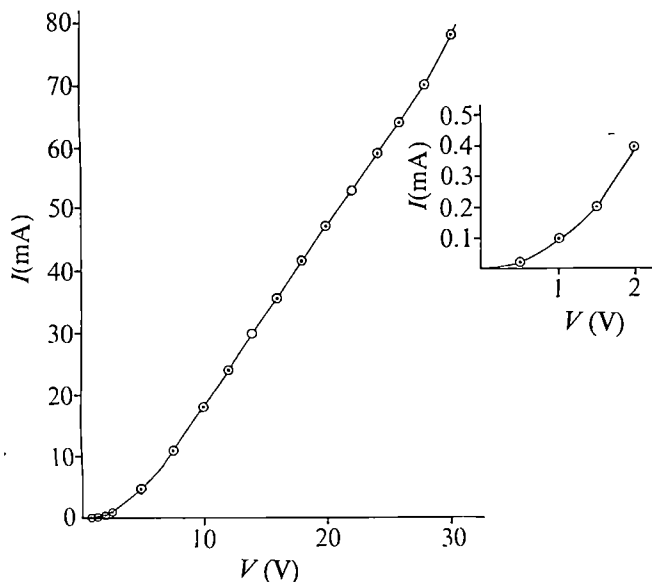


Fig. 1 Measured capacitance ( $C$ ,  $\circ$ ) and electrical conductivity ( $\sigma$ ,  $\Delta$ ) as a function of frequency ( $f$ ) of the magnetisable liquid.

used as the source of a relatively homogeneous field. The relative permeability of the liquid was found to be  $\sim 1.2$  in other measurements (not reported here). It may, therefore, be assumed that the liquid and probe hardly influence the homogeneity of the applied magnetic field. For inhomogeneous fields, a small horseshoe magnet was fitted around the measurement tube, and produced a magnetic field in the liquid varying from zero to 600 gauss.

Homogeneous fields were found to have a negligible effect on the resistance of the magnetisable liquid, but large changes in the current could be effected in the low-voltage region ( $< 5$  V) by appropriately aligning the position of the horseshoe magnet. Thus, for example, at 0.5 V the current increased by  $2 \mu\text{A}$  from its value of  $40 \mu\text{A}$  in the absence of a field, representing a 5% overall change.

Fig. 2 d.c. Voltage-current characteristics of the magnetisable liquid.



It was also established that a homogeneous field produced changes in the capacitance as measured on the a.c. bridge. At 750 Hz, for example, the change in capacitance as a function of magnetic field applied perpendicularly to the conduction path is shown in Fig. 3. At 270 gauss, the increase in capacitance is  $\sim 0.3\%$ . A magneto-resistance effect is also noted but the effect is considerably weaker.

Figure 1 suggests that the unusually large measured capacitances are not bulk values for the liquid but arise from a polarised layer on the surface of the porous electrodes, (or an analogous effect in the colloid), the resistance seems to represent a bulk property. Accordingly the specific resistivity (or conductivity) of the liquid may be estimated, whereas no simple derivation of the dielectric constant can be obtained from the capacitance measurement.

If the liquid is represented in a simplified model, with the electric path between the two electrodes having a resistance  $r$  and series capacitance  $C_s$ , then the admittance across the probe is

$$Y_s = \frac{1}{r + (1/j\omega C_s)} = \frac{r - (1/j\omega C_s)}{r^2 + (1/\omega^2 C_s^2)} \quad (1)$$

The General Radio Bridge, however, measures a capacitance  $C$  as if it were in parallel with a resistance  $R$ . In such a configuration, the admittance would be

$$Y_p = \frac{1}{R} + j\omega C_p \quad (2)$$

Comparing the real and imaginary parts of equations (1) and (2),

$$\frac{1}{R} = \frac{\omega^2 C_s^2 r}{\omega^2 r^2 C_s^2 + 1} \quad (3a)$$

and

$$C_p = \frac{C_s}{1 + \omega^2 r^2 C_s^2} \quad (3b)$$

at high frequencies such that

$$\omega^2 r^2 C_s^2 \gg 1$$

$$R \approx r = \frac{1}{S} \text{ (constant)} \quad (4a)$$

and

$$C_p \approx \frac{1}{\omega^2 r^2 C_s} \quad (4b)$$

Equations (4a) and (4b) give a satisfactory description of the experimental results. Above 80 Hz, the measured conductivity  $S$  remains almost constant, while the capacitance varies as  $\omega^{-2}$  (or  $f^{-2}$ ).

Information about the characteristic dielectric properties of the liquid are not easily obtained from the capacitance measurements because of the complex dependence of the polarisable layer on the specific nature of the electrodes used, and it was found that different electrodes yield different values of capacitance. It is hoped that a better understanding of the polarisable layer or its equivalence will be gained in future investigations.

Support for the above model is provided by the voltage-current characteristic. The large time constants and correspondingly large resistances observed at low voltages indicate high values of the layer capacitance. When the voltage increases, however, there seems to be a gradual breakdown effect, and the measured d.c. resistance approaches its a.c. value of  $330 \Omega$ . Under a.c. conditions the proposed layer behaves as a coupling capacitance  $C_s$  according to equation (1), and therefore a similar



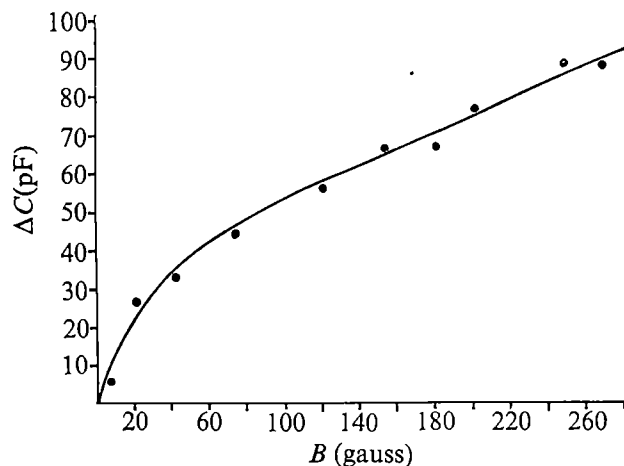


Fig. 3 Increase of capacitance ( $\Delta C$ ) as a function of applied magnetic field  $B$  for the magnetisable liquid.

bulk resistance is measured. The a.c. measurements of capacitance made use of small signals and consequently no breakdown effect was encountered.

The cross effects of magnetic fields on the voltage-current characteristics are like the capacitance attributed here to the proposed polarised layer. This is consistent with the observation that the capacitance changes more strongly with magnetic field than does the conductivity in the a.c. measurements. Moreover, at high voltages the influence of the magnetic field on the d.c. measurements drops to zero because of the presumed breakdown of the layer. It is proposed that inhomogeneous fields exert a stronger influence on the voltage-current characteristic at low voltages than do homogeneous fields because they can more easily produce irregularities in the layer which might thereby be locally 'punctured'.

Different electrodes and ferrofluids might increase the magneto-electric effect. It is believed that this phenomenon is affected by the saturation magnetisation of the liquid, as well as by the solvent and dilution of the colloid used. If the effect can be sufficiently enhanced, possible applications may be found in energy conversion and signal processing. From a theoretical viewpoint, we believe that the observed phenomena in magnetisable liquids provide a new means of probing layer effects in colloids and might help provide further insight into the electrical behaviour of colloids in general.

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can be prepared<sup>2</sup>, such values are insufficient to make it of use as an electrolyte in solid state batteries at room temperature. There is, however, considerable interest in producing solid electrolytes, based on AgI combined with organic cation iodides to give high silver-ion conductivity at room temperature<sup>4-9</sup>. We report here on the conductivities of representatives of three hitherto unexamined classes of compounds, AgI-selenonium iodide, AgI-telluronium iodide and AgI-bis-sulphonium diiodide.

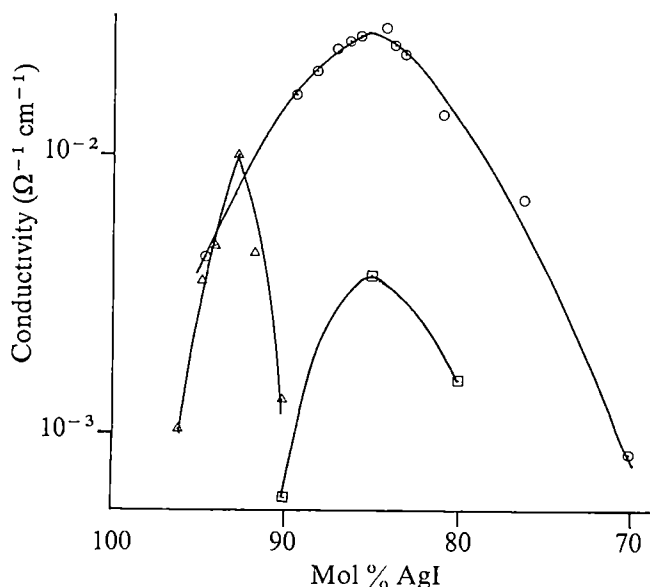
As a representative of the AgI-SeI system,  $(\text{CH}_3)_3\text{SeI}$ , prepared from  $(\text{CH}_3)_3\text{Se}$  and  $\text{CH}_3\text{I}$  (ref. 10) was mixed with varying amounts of AgI to produce samples containing 90, 85 and 80 mol % AgI. The powdered samples were suspended in distilled water and heated to  $90 \pm 5^\circ\text{C}$  for a total of 16 h. The solid product was filtered off and washed with ethanol and dry ether, before being pressed as a three-layer pellet, the outer layers of which were silver powder mixed with powdered electrolyte. The conductivities for each of the three samples at this stage were  $< 10^{-5} \Omega^{-1} \text{cm}^{-1}$  at  $21^\circ\text{C}$ . The pellets were then heated in nitrogen to  $140 \pm 2^\circ\text{C}$  for 9 h, after which the conductivities at  $21^\circ\text{C}$  reached the values shown in Fig. 1. For comparison, we show also results for AgI- $(\text{CH}_3)_3\text{SI}$ . The conductivity for the Se-containing electrolyte was  $4 \times 10^{-3} \Omega^{-1} \text{cm}^{-1}$  at 85 mol % AgI, compared with  $2.7 \times 10^{-2} \Omega^{-1} \text{cm}^{-1}$  for the S-containing electrolyte at the same mol %. A ten-fold deterioration in conductivity was observed over a period of a few weeks, which may have been caused by the presence of moisture<sup>11</sup>.

The loss of conductivity makes the AgI- $(\text{CH}_3)_3\text{SeI}$  system unsuitable for battery use, although other Se-containing systems may be more suitable. The first member of the AgI-TeI system that we have studied is  $\text{Ag}_{5.5}(\text{CH}_3)_3\text{TeI}_{6.5}$  which was prepared by grinding together appropriate amounts of  $(\text{CH}_3)_3\text{TeI}$ , prepared from  $(\text{CH}_3)_3\text{Te}$  and  $\text{CH}_3\text{I}$  (ref. 10), and AgI, pressing into a three-layer pellet and heating to  $140 \pm 10^\circ\text{C}$  for 7 h. The conductivity was  $< 10^{-5} \Omega^{-1} \text{cm}^{-1}$ , making it unsuitable for battery use.

The relatively poor properties of these Se- and Te-containing electrolytes, when taken in conjunction with their high toxicities and the consequent difficulties that arise in handling them indicate that such systems are unlikely to be exploited commercially for some considerable time.

We have found, however, that the performance of bis-sulphonium diiodides is altogether more encouraging. 1,3-bis(dimethylsulphonium)propane diiodide was prepared by the

Fig. 1 Conductivities of solid electrolyte systems incorporating AgI and (a)  $(\text{CH}_3)_3\text{SeI}$  ( $\square$ ); (b)  $(\text{CH}_3)_3\text{SI}$  ( $\circ$ ); (c)  $(\text{CH}_3)_3\text{S}^+(\text{CH}_2)_3\text{S}^+(\text{CH}_3)_2\text{I}^-$  ( $\Delta$ ) as a function of mol % AgI.



## Search for new silver-ion solid electrolytes for use in batteries

THE  $\alpha$ -phase of AgI, which is stable above  $146^\circ\text{C}$ , has a silver-ion conductivity of  $1 \Omega^{-1} \text{cm}^{-1}$  at  $150^\circ\text{C}$  (ref. 1), which would make it a suitable solid electrolyte for high temperature batteries. The room-temperature conductivity of the stable phase of AgI is disappointingly low ( $10^{-6} \Omega^{-1} \text{cm}^{-1}$ ) and although samples with conductivities approaching  $3 \times 10^{-4} \Omega^{-1} \text{cm}^{-1}$

method of Protiva, Jilek and Exner<sup>12</sup>, ground up with the correct amount of AgI and pressed into three-layer pellets, the outer layers comprising a mixture of silver powder and powdered electrolyte. Pellets containing 96, 95, 94, 93, 92 and 90 mol % AgI were prepared in this way, these proportions being selected because the conductivity maximum for a dication would be expected to be within this region, by analogy with the dimethonium results<sup>4</sup>. The pellets were heated in nitrogen at  $120 \pm 2^\circ \text{C}$  for 66 h, after which their conductivities at  $21^\circ \text{C}$  were measured. The values are presented in the Fig. 1. The conductivity is  $1.0 \times 10^{-2} \Omega^{-1} \text{cm}^{-1}$  for 93 mol % AgI–7 mol %  $(\text{CH}_3)_2\text{S}^+(\text{CH}_2)_3\text{S}^+(\text{CH}_3)_2 2\text{I}^-$ , which remained constant over a period of several weeks. This stability, which contrasts with the results for Se- and Te-containing electrolytes, makes this system a plausible choice for use in a solid state cell. Conductivity results for the systems  $\text{AgI}-\text{R}_2\text{S}^+(\text{CH}_2)_m\text{S}^+\text{R}_2 2\text{I}^-$  and  $\text{AgI}-(\text{CH}_2)_n\text{S}^+(\text{CH}_2)_m\text{S}^+(\text{CH}_2)_n 2\text{I}^-$ , and their use in solid-state electrochemical cells will be reported later.

We thank Mallory Batteries (UK) Ltd for support, Mr M. I. Needham for NMR spectra and Mr C. L. Groves for preparing  $\text{CH}_3\text{S}(\text{CH}_2)_3\text{SCH}_3$  from which the dication was prepared.

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## Fossil hominid femora and the evolution of walking

THE evolutionary origins of human bipedalisms are still poorly understood, but recent discoveries of fossil hominid femora in Africa reveal some interesting details. Especially important are several new fossils discovered by the expedition led by R. E. F. Leakey to East Rudolf, Kenya<sup>1-9</sup>. The purpose of this paper is to find the morphological and functional affinities among higher primates of these and other fossil femora in the hope of revealing more about the nature of early hominid locomotion.

The hominid fossils analysed here include two from Swartkrans, South Africa (SK 82 and SK 97), discovered in 1949 by Broom and Robinson<sup>10</sup>, dated variably between 1.5 and 3 Myr<sup>11-15</sup>, and classified as *Paranthropus* or *Australopithecus robustus*; and three from east of Lake Rudolf discovered by R. E. F. Leakey's expedition in 1972<sup>3-9</sup> and classified as *Australopithecus* sp. indet. (KNM-ER 1503, dated between 1.8 and 2.6 Myr) and *Homo* sp. indet. (KNM-ER 1472 and 1481c, both dated between 1.6 and 3 Myr)<sup>16</sup>.

The comparative sample consists of either the left or right femur from 57 *Homo sapiens*, 42 *Pan troglodytes*, 16 *Pan paniscus*, 66 *Gorilla gorilla*, and 34 *Pongo pygmaeus*.

Ten measurements are used to represent the shape of the

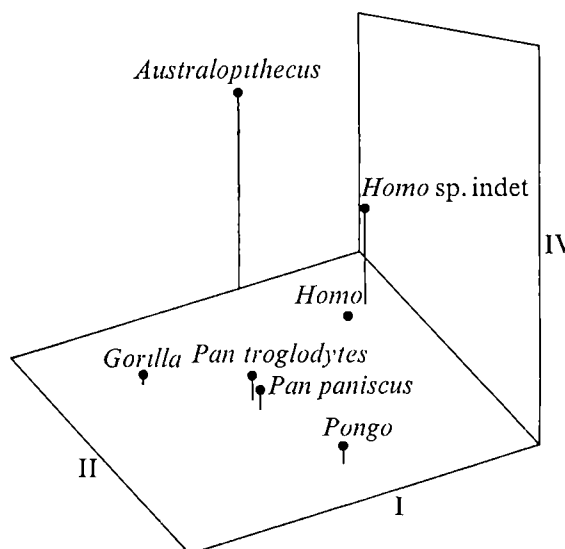
proximal femur. These are the vertical head diameter, vertical neck diameter, anteroposterior neck diameter, transverse shaft diameter, anteroposterior shaft diameter, the projected distance from the centre of the head to the lateral surface of the greater trochanter measured perpendicular to the shaft axis, neck length, two measurements from the inferior border of the lesser trochanter: one to the superior surface of the neck, and one to the centre of the head, and the projection of the greater trochanter above the neck. A full description is given elsewhere<sup>17</sup>.

These data were analysed using canonical variates analysis<sup>18</sup>. The measurements are first adjusted for possible allometric effects, using average within-taxon allometry coefficients between each measurement and a standard size variable<sup>19-21</sup>. They are then converted to ratios of the size reference variable for each specimen, producing dimensionless shape variables which are relatively independent of factors of size. Canonical analysis is performed by entering each taxon as a separate group. All of the hominid fossils are entered as a single group in the original calculations.

Figure 1 displays the results by plotting the first, second and fourth canonical variates, which together account for 82.9% of the total variance (47.3%, 30.9%, and 4.7% for axes 1, 2, and 4, respectively). The third canonical variate is not plotted because it merely acts to separate *Pan* from the other hominoids. The fossil hominid femora are quite variable, which might be expected considering the changes in locomotor adaptation in human evolution and their possible time span. The three femora that have been classified<sup>3-10</sup> as *Australopithecus* (SK 82, SK 97, and KNM-ER 1503) are separated from *Homo sapiens*. The two fossils classified as *Homo* (KNM-ER 1472 and 1481c) are closer to modern *Homo sapiens*.

On the first canonical variate all of the hominids are separated from the rest of the primates. The variables with the highest correlation with this variate include the neck length and the distance that the greater trochanter projects above the neck. The neck is very long in all of the fossil femora and moderately long in modern *Homo*. The human greater trochanter is lower than in the other hominoids, but in all of the early hominids the greater trochanter barely projects at all. Presumably the differences in the projection of the greater trochanter reflect differences in the function of the gluteus medius, gluteus minimus, and piriformis muscles which attach on the greater trochanter. The two glutei muscles act primarily as abductors in bipedal

Fig. 1 Distribution of group centroids and fossils on the first, second, and fourth canonical variates which account for 82.9% of the total variance



hominids—their pull is essential to the lateral support system in the human hip<sup>22</sup>. The length of the femoral neck is another feature which is associated with the abductor mechanism of the bipedal hominid hip, since the length of the neck is related to the length of the abductor lever arm. The very long femoral neck of the early hominids may imply that they had mechanically efficient lateral support mechanisms in their hips<sup>23</sup>.

The second canonical variate maximises the separation of *Pongo* from the other hominoids, and the variables with high correlations with this axis are those which describe the unique morphology of the orangutan hip. The third variate separates the two species of *Pan* from the rest of the primates. It is on the fourth variate that the fossil hominid femora differ significantly from modern *Homo*. Traits most closely associated with this axis are the same as those associated with the first variate, except the head size is also heavily weighted. In the three hominid fossils classified as *Australopithecus* (KNM-ER 1503, SK 82, SK 97) the head diameters are uniquely small, but in the two classified as *Homo* (KNM-ER 1481c and 1472) they are more similar to extant hominids. As a consequence of this resemblance and others, the two early *Homo* femora lie nearer to the modern *Homo* group on the fourth canonical axis, with the australopithecine femora in an isolated position.

Other axes account for the small residual variances, but offer no other useful interpretation.

Figure 2 compares the relative size of each dimension in the two most complete fossil hominids (KNM-ER 1481c and SK 97) and *Homo sapiens*. The significant fact revealed by this figure is the similarity between the two hominid fossils in many traits and how different they are in the same way from *Homo sapiens*. The only conspicuous differences between the two fossils are in their relative head size and relative anterior-posterior shaft thickness. This result contrasts with the preliminary visual assessment and many univariate comparisons concerning KNM-ER 1481c.

These results can be related to what is known of australopithecine hip-joint mechanics. Although these fossil hominids were bipedal, their pelvic girdles are morphologically different from modern humans in having laterally splayed iliac blades, small acetabulae, and long femoral necks. Lovejoy *et al.*<sup>23</sup> give a biochemical explanation of these differences suggesting that the evolutionary changes between the hip of the australopithecines and *Homo sapiens* is a result of encephalisation: the increase in cranial size in human evolution meant a concomitant increase in size in the birth canal. The intermediate position of the two East Rudolf femora classified as *Homo* could be related

to the intermediate cranial capacity apparent in that population.

Although the present study deals with only one half of a joint and not with a total functional unit, the proximal femur is an integral part of hominid locomotion. Its morphology is highly correlated with that of the pelvis and other parts of the hindlimb. Unfortunately, there are no published reports of complete pelvises associated with femora in the early hominid fossil collections except for Sts 14 from Sterkfontein, South Africa, but a large part of its proximal femur is missing, including the head and most of the neck.

The findings do support the idea expressed by Leakey and others<sup>3-9</sup> that there were at least two distinctive forms of hominids with one more closely related to the later hominids than the other. The distinctively long neck of all of the early hominids indicates that the abductor lever arm was probably long which would yield a favourable bio-mechanical arrangement for the lateral support system in the hip which is necessary for human walking. The differences between the fossils classified as *Homo* and *Australopithecus* may imply that the hip joint mechanics were different. A major morphological difference is the size of the femoral head. If Lovejoy *et al.*<sup>23</sup> are correct in their assessment of early hominid hip biomechanics, the large femoral heads might be a result of a widened birth canal. Whether or not there was more than one kind of gait among the early hominids is still uncertain.

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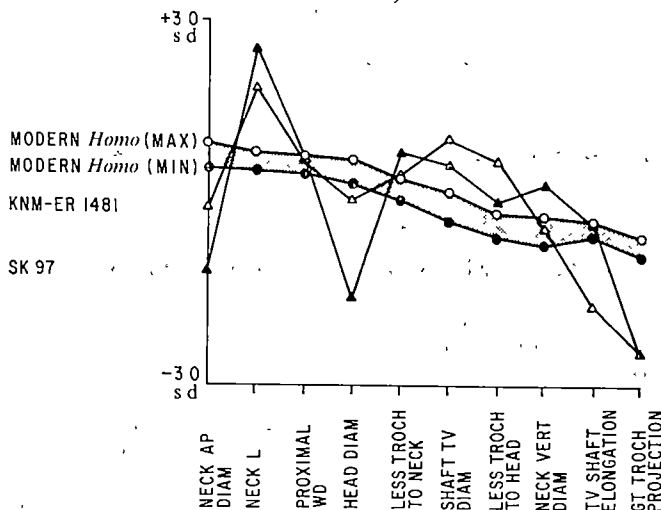
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Fig. 2 Comparison of shape variables between *Homo sapiens* and the two most complete hominid proximal femora (KNM-ER 1481c and SK 97).



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## Hypothesis for origin of planktonic patchiness

ALTHOUGH environmental heterogeneity can contribute to the observed patchiness in oceanic planktonic populations, biological interactions between phytoplankton and herbivorous copepods can lead to similar patterns. The phenomenon of spontaneous pattern generation even in a homogeneous environment through the interplay of reaction and movement is a very general property of the class of mathematical 'reaction-diffusion' equations used to model such systems, and there are several ways in which such patterns may occur. The result may be either spatiotemporal patterns<sup>1</sup> or time-independent spatial patterns. Usually one thinks of diffusion as damping inhomogeneities, and a hypothesis put forward by Steele<sup>2</sup> essentially relies on a balance reached between the dehomogenising aspects of local interaction and the homogenising influence of diffusion to produce pattern. On the other hand, it has also been suggested<sup>3-6</sup> that in some conditions diffusion can destabilise an otherwise stable interaction (in the manner originally suggested by Turing<sup>7</sup>) to produce pattern. We have explored<sup>5</sup> this further in a nonlinear analysis that describes the final stages of pattern formation, and the resultant calculations provide some insights into the spatial scale of patterns which might arise in this way.

The results that we describe depend on two hypothesised properties of the full equations: an autocatalytic effect in phytoplankton density which is attributed to a reduced efficiency of herbivory as phytoplankton density increases (a Holling<sup>8</sup> Type 2 functional response), and differential dispersal rates which favour higher herbivore motility. Both assumptions may be justified on theoretical grounds (the work of Holling for the first, and the greater mobility of herbivores by comparison with phytoplankton for the second), but the data are at present insufficient to provide the real test.

The relevant equations<sup>5</sup> thus take the form

$$\partial P/\partial t = aP + eP^2 - bPH + \nabla \cdot (\mu \nabla P)$$

$$\partial H/\partial t = cPH - dH^2 + \nabla \cdot (\nu \nabla H)$$

where  $P$  is phytoplankton density,  $H$  herbivore density,  $t$  time, and  $\mu$  and  $\nu$  are species-specific diffusion coefficients.  $a$ ,  $b$ ,  $c$ ,  $d$  and  $e$  are all assumed positive. The assumption of passive diffusion is made for simplicity only; more complicated movement patterns can also lead to diffusive instability.

Assuming homogeneity of dispersal rates and restricting attention to one space dimension, we obtain the modified equations

$$\partial P/\partial t = aP + eP^2 - bPH + \mu(\partial^2 P/\partial x^2)$$

$$\partial H/\partial t = cPH - dH^2 + \nu(\partial^2 H/\partial x^2)$$

In the linearised study of small disturbances, one exposes the system to small amplitude perturbations of the initial form

$$P = c_1 \cos qx, H = c_2 \cos qx$$

(Rather general disturbances can be Fourier synthesised from such perturbations.)

The system is seen to have a spatially uniform stable equilibrium

$$P = (ad)/(bc - ed), H = (ac)/(bc - ed)$$

provided  $bc - ed > 0$ ,  $c > e$ , and

$$R = \nu/\mu < [1/\{\sqrt{(b/d)} - \sqrt{[(b/d) - (e/c)]}\}]^2 = R_{cr}$$

We note that  $R_{cr} > 1$ .

When  $R > R_{cr}$ , the local activation effect due to the autocatalytic term interacts with the long range inhibition<sup>9</sup> to destabilise the uniform state. For  $R$  slightly greater than  $R_{cr}$ , a perturbation will serve to destabilise if its initial wavelength is approximately equal to  $1/q_{cr}$ , where

$$q_{cr} = [\sqrt{(b/d)}/\sqrt{[(b/d) - (e/c)]}] - 1$$

By a combination of successive approximations and multiple time scales, we have shown<sup>5</sup> that for  $R$  slightly greater than  $R_{cr}$ , the uniform state is replaced by a new steady state in which plant and herbivore are more concentrated in certain regions (with an overall elevation in mean herbivore density). The limit to the growth of the perturbation is a result of the distortion of the initial periodic spatial distribution due to nonlinear interactions.

When two-dimensional effects are introduced, we expect (as happens in the discrete spatial case) that perturbations of large enough amplitude will be shown to destabilise systems which are stable to small perturbations, and that generally the new steady state which arises from instability of the uniform state will either be net-like or spot-like, depending on a certain combination of parameters. This type of analysis is valid for initially homogeneous regions that are large compared with  $1/q_{cr}$ .

We emphasise that we offer here only one mechanism for generating pattern—not necessarily the principal one. In eventually distinguishing between hypotheses, however, it should be helpful to do the kind of nonlinear analysis we have carried out. To our knowledge this has not previously been done for any of the hypotheses.

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## Intercalary regeneration in imaginal wing disk of *Drosophila melanogaster*

In this paper we show for the first time that the developmental capacity of imaginal disk cells from *Drosophila* can be altered by appropriate grafting operations. The appendages of amphibians and immature arthropods, and the imaginal disks of holometabolous insects, are capable of undergoing pattern regulation when parts are removed. (Pattern regulation is used here to describe the alteration of cell fates in response to an



abnormal situation in a developing system; we include under this term both regeneration and duplication.) Regulation then occurs during growth of the tissue by cell division, a process termed epimorphosis<sup>1</sup>. A characteristic feature of epimorphic regulation which has recently been recognised, is the different regulative responses shown by complementary pieces. For example when an imaginal disk of *Drosophila* is bisected and allowed to grow for some time, one of the two fragments usually regenerates the missing parts, while the other undergoes duplication of the presumptive pattern already present<sup>2</sup>. Similarly, whereas the stump of an amputated amphibian or cockroach limb can regenerate distally, the amputated distal part, if kept alive by grafting to a host animal, can also undergo pattern regulation by forming the distal pattern elements, thereby duplicating itself<sup>3-6</sup>.

It has been suggested<sup>1,2</sup> that the complementarity between regeneration and duplication can be understood if it is assumed that new positional values<sup>1</sup> can be generated during growth only in a fixed sequence, a proximal-to-distal sequence in the case of a regenerating limb<sup>7</sup>. The spatial pattern of differentiation thus could be established by a temporal sequence of events occurring autonomously in the dividing cells as the tissue grows out from the cut edge. Under certain circumstances, however, it is clear that the developmental fate of a cell is a function not only of its history but also of its position with respect to other cells. For example, during regeneration from the stump of a cockroach leg or during duplication from the distal leg fragment, the newly produced cells attain positional values only more distal than those of their progenitors. But when the distal part of a leg is grafted to a stump at a more proximal level, intercalary regeneration occurs between the graft and the stump, and the cells produced by the graft can attain more proximal positional values<sup>8</sup>. The results of other graft combinations indicate that the graft can have a similar effect on the development of the stump<sup>9</sup>. Similar results

Fig. 1 Simplified fate map of the imaginal wing disk of *Drosophila melanogaster*, showing only the markers which are genotypically recognisable in our combinations (abbreviations as in Table 1). From Bryant<sup>13</sup>. The positions of the cuts used to generate the fragments are also indicated.

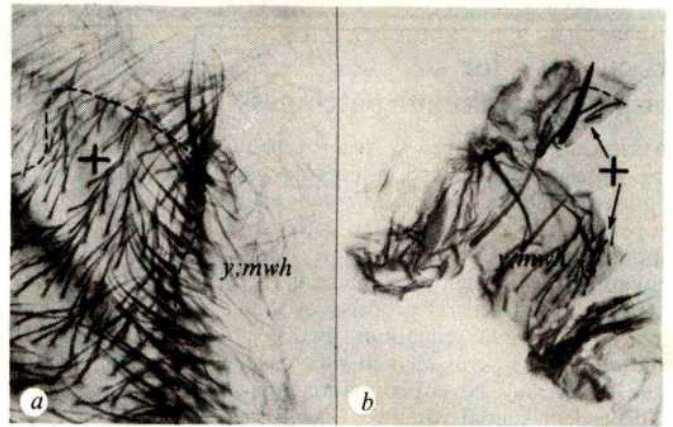
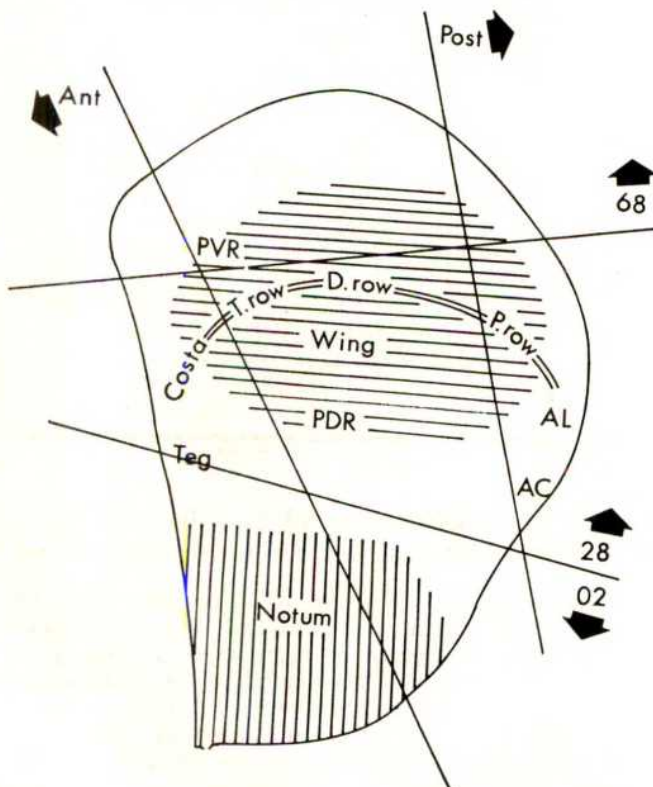


Fig. 2 a, Mosaic wing spread and posterior row produced by a 02/28 combination. The arrangement of posterior row bristles of alternate genotype is similar to that found in mosaics produced *in situ*<sup>19,20</sup>. b, Mosaic notum produced by a +02/y;mwh 68 combination.

have been reported for the legs of other insects<sup>10</sup>, for regenerating amphibian limbs<sup>11</sup> and for developing chick limbs<sup>12</sup>.

When a fragment of a *Drosophila* imaginal disk is transplanted to a mature host larva, it undergoes metamorphosis with the host, and the cells of the fragment differentiate those structures which they would have formed *in situ*. This has made possible the establishment of detailed fate maps of several of the imaginal disks, and that for the wing disk is shown in Fig. 1. If, however, the fragment is cultured in an adult abdomen for several days (to allow for growth) before transfer to a host larva for metamorphosis, it can either regenerate the missing parts or duplicate the presumptive pattern already present<sup>2,14</sup>. In the case of the wing disk, it was possible to localise a point in the disk which defines the direction in which regeneration usually occurs; fragments with cut surfaces facing away from this point show regeneration, whereas those with cut surfaces facing in the direction of this point show duplication<sup>13</sup>.

In our first experiments, we studied the interaction of the presumptive notum (02 piece; Fig. 1) with the presumptive wing (28 piece). When cultured alone, the 28 piece frequently regenerates the missing notum whereas the 02 piece undergoes duplication<sup>13</sup>. Individual 02 pieces of one genotype (wild-type *Oregon-R* or *y;mwh*) were mechanically mixed with individual 28 pieces of the other genotype. Mixing was accomplished with two tungsten needles in a drop of buffered Ringer's solution, the two fragments being folded together about 20 times so that they formed a coherent mass. The combinations were transplanted to the abdomens of fertilised female adults, where they remained for 7 d. They were then dissected out of the adult hosts and re-injected into larval hosts for metamorphosis; the larger cultured combinations had to be cut into two or more fragments before they could be transplanted to larval hosts. The differentiated implants were recovered from the metamorphosed hosts and mounted between coverslips for scoring of the structures of each genotype. Control combinations were similarly produced from two 02 or two 28 pieces of different genotypes. The results show that many structures which are never produced by isolated 02 fragments<sup>13</sup> or 02 combinations (02/02, Table 1) are produced with a high frequency by cells from the 02 piece when in combination with cells from a 28 piece (02/28, Table 1). The 28 piece could simultaneously regenerate the notum, but this is not remarkable since it occurs in cultured isolated 28 pieces<sup>13</sup> or 28 combinations (28/28, Table 1). Many of the patterns produced by the 02/28 combinations were mosaic, containing cells of both genotypes (Fig. 2a) and patterns were frequently duplicated.

In this experiment all of the positional values regenerated



**Table 1** Numbers of cases in which specific structures are formed from combinations of wing disk fragments cultured for 7 d in adult hosts followed by transfer to larvae for metamorphosis

	02/28 <i>n</i> = 29		02/02 Control <i>n</i> = 12	28/28 Control <i>n</i> = 13	02/68 <i>n</i> = 27		68/68 Control <i>n</i> = 10	Ant/Post <i>n</i> = 21		Ant/Ant Control <i>n</i> = 12	Post/Post Control <i>n</i> = 11
	From 02	From 28			From 02	From 68		From Ant	From Post		
Notum	29	24	12	11	27	6		21		12	
Tegula (Teg)	11	17	3	12	14	7		19		12	
Costa	4	21		13	8	9		14		9	
Triple (T.) row	2	26		13	6	7		11		1	
Double (D.) row	8	25		12	7	9	5	15	12		
Posterior (P.) row	11	26		11	10	12	4	6*	15		8
Alar lobe (AL)	6	24		10	6	11	2	1*	13		11
Axillary cord (AC)	6	21		8	3	9	1	1*	6		10
Proximal radius (dorsal) (PDR)	9	22		12	12	1		14			
Proximal radius (ventral) (PVR)	5	16		9	2	10	7	12	1*		
Wing	20	29		13	13	27	10	19	20	1	11

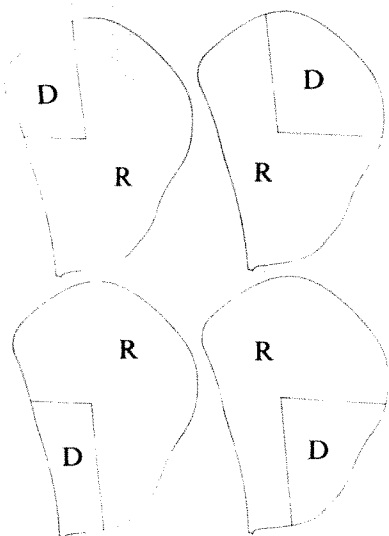
\*Regeneration across the anterior-posterior compartment boundary.

y; *mwh* and wild type were used in reciprocal combinations and the results combined. 02, 23, 68, Ant and Post represent the fragments illustrated in Fig. 1. Structures not found in control combinations of identical fragments but appearing in combinations of two different fragments are shown in bold. Combinations producing extensive allotypic structures following transdetermination are not included in these results.

by the 02 piece are already present in the 28 piece with which it is mixed; this suggests that the positional values of the cells derived from the 02 piece are merely being copied from adjacent cells from the 28 piece. In a second experiment, we tested for regeneration of structures which are not normally produced by either fragment, even when cultured. We mixed 02 pieces (presumptive notum) with 68 pieces (presumptive ventral wing blade, ventral hinge, and pleura). Both fragments showed duplication when cultured separately<sup>13</sup>, but in combination they showed intercalary regeneration of many of the structures not normally produced by either of them (02/68, Table 1). Again, the patterns produced were frequently mosaic and often duplicated (Fig. 2b). In six cases, cells from the 68 fragment produced parts of the notum, usually derived from the opposite end of the disk (Fig. 1).

We have also tested for intercalary regeneration in the anterior-posterior direction in the wing disk (Table 1). Fragments totally within the anterior compartment<sup>15</sup> were mixed with fragments totally within the posterior compartment (Fig. 1). Each of these fragments is taken from the edge of the disk, and is therefore expected to duplicate<sup>13</sup>; accordingly, the control combinations of anterior and posterior fragments (Ant/Ant and Post/Post, Table 1) produced only the structures expected from the fate map.

**Fig. 3** Regenerative behaviour of sectors of the wing disk. In each case, the complementary 90° and 270° sectors were cultured in adult abdomens for 7 d before transfer to host larvae for metamorphosis. R, Regeneration; D, duplication.



When mixed together, however (Ant/Post, Table 1), they showed intercalary regeneration of intermediate structures as in the 02/68 combination, but in most cases each of the fragments in the combination regenerated only those structures within its own (anterior or posterior) compartment, so that the frequency of mosaic patterns was much lower than in the previous experiments. Regeneration of specific structures in the other compartment occurred in nine cases, but this might be an underestimate of the true frequency of regeneration across the compartment border since anterior and posterior wing blade are indistinguishable in implants, and regeneration of one from the other cannot be detected. In our first and second series of experiments the wing-notum, proximal-distal and dorsal-ventral compartment boundaries were clearly transgressed, but in the case of the dorsal-ventral boundary we found some evidence for the maintenance of compartmentalisation since the genotypic boundary in mosaic patterns often coincided with the wing margin bristles or hairs, as reported previously<sup>16</sup> (Fig. 2a).

The occurrence of intercalary regeneration provides a simple explanation for the hitherto puzzling behaviour of certain wing disk fragments when cultured alone. The regulatory behaviour of segments of this disk could be accounted for, as described above<sup>13</sup>, on the basis of the direction faced by the straight cut edges. In the same culture conditions, however, each of four 90° sectors of the wing disk duplicates while the complementary 270° sectors regenerate (P.J.B., unpublished, and see Fig. 3); this cannot be accounted for in terms of the predicted regulatory behaviour of the individual cut edges of the sectors. We have also found, however, that in the first few days of culture, the two cut edges of these fragments heal together (unpublished results). If we assume that intercalary regeneration then occurs between the levels of the two fused edges, the results are easily explained. Intercalary regeneration can also account for the ability of imaginal disks to produce a normal pattern after more than half of the cells are killed by X rays (our unpublished results).

Our results also offer a way of understanding pattern reconstruction by dissociated imaginal disks. Mosaic patterns of the kind illustrated in Fig. 2a and b have been produced in many experiments where genetically marked disks are dissociated into cells and cell clumps, and reaggregated before culture and metamorphosis<sup>16-18</sup>. If mosaic patterns can develop on a large scale by intercalary regeneration between fragments from opposite ends of the disk, it seems logical to assume that the same process operating on a smaller scale could account for pattern reconstruction in dissociated and reaggregated disks. This kind of interaction

between cells obviates the need to assume that extensive cell migration<sup>17</sup> is necessary for pattern reconstruction.

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## Are morphogenetic tissue interactions mediated by transmissible signal substances or through cell contacts?

TRANSMISSION of morphogenetic signals between embryonic cells is a special form of intercellular communication which may involve diffusible signal substances<sup>1,2</sup> or intercellular contacts<sup>3,4</sup>. We have previously presented evidence that the induction of metanephric kidney tubules is dependent on cell contacts between the interacting tissues rather than on diffusible signals<sup>5,7</sup>. Several embryonic and neoplastic tissues possess an inductive effect when tested against metanephric mesenchyme<sup>8-11</sup>. If different inductors release an extracellular, transmissible signal substance, the restriction of induction by interposed filters with varying pore sizes should be independent of the type of inductor tissue, assuming that there is no major difference in size between signal substances. If cell contacts and thus ingrowth of cytoplasmic processes into the filter are required, however, there may be differences between the transfilter inductive capacity of various inductor tissues depending on their ability to send cytoplasmic processes through the filter. That is now shown to be the case, in a comparison of the spinal cord and the mesenchyme of embryonic salivary gland as inducers of metanephric mesenchyme.

Direct combination of salivary mesenchyme with metanephric mesenchyme resulted in the formation of well shaped, coiling tubules with high mitotic activity in all 6 cases, which indicates that salivary mesenchyme like spinal cord is a potent inductor in direct combination. When a Millipore 'TA' filter (pore size 0.8  $\mu$ m, thickness 25  $\mu$ m) was interposed between the tissues only 1 of 7 explants showed any tubule response. In the other explants, the metanephric mesenchyme spread out on the filter with good viability but without tubule formation. When spinal cord was used as an inductor, transfilter tubule formation was regularly seen<sup>14</sup>.

Nuclepore filters with pore sizes between 0.2 and 0.6  $\mu$ m allowed the passage of the inductive signal from the spinal cord to the responding metanephric mesenchyme but, in

identical conditions, prevented passage of the signal from the salivary mesenchyme (Table 1). When filters with a nominal pore size of 3.0  $\mu$ m separated the salivary and kidney mesenchymes, tubules were observed in 8 of 14 explants. These filters, however, allowed the passage of whole metanephric cells through the filter and kidney tubules were often seen below the filter intermingled with salivary cells.

Light microscopy of glutaraldehyde fixed specimens with salivary mesenchyme as inductor showed the presence of cytoplasmic material throughout the 3.0- $\mu$ m pores, and variable amounts of stainable material in the 0.6- $\mu$ m pores, and this was confirmed by transmission electron microscopy. This contrasts with the abundant penetration of spinal cord material into even smaller pores<sup>6</sup>.

Scanning electron microscopy of filters where tissues were grown on one side only, demonstrated abundant cytoplasmic penetration through 3.0- $\mu$ m pores when either salivary mesenchyme or spinal cord was cultured for 48 h (Fig. 1a). Processes from the spinal cord were seen to penetrate even the smallest pores (0.1  $\mu$ m) whereas salivary mesenchyme never protruded through pores of twice that size (Table 1). Four out of seven explants with salivary mesenchyme cultured for 48 h on the lower surface of a filter with 0.6  $\mu$ m pores showed penetration of cytoplasmic processes in restricted areas. In contrast, there were abundant processes when spinal cord was cultured in identical conditions (Fig. 1b). When metanephric mesenchyme was cultured on top of various Nuclepore filters, cell processes (and whole cells) penetrated to the lower surface only through the largest pores.

Thus the two inductors, spinal cord and salivary mesenchyme, gave identical induction responses in direct combination with the responding metanephric mesenchyme, but showed marked differences in inducing capacity when separated from the responder by a filter. While spinal cord was occasionally able to pass an inductive signal through 0.1  $\mu$ m pores and did so regularly through larger channels, salivary mesenchyme required pores ten times as large to send the morphogenetic signal to the responding mesenchyme. If the message is carried by transmissible molecules, the results imply that the signal molecules are markedly different in the two inductor tissues, and that the 'salivary factor' is trapped by  $\sim$  0.6- $\mu$ m pores. Measurements of the passage of various molecules through Nuclepore filters have not lent support to such an idea, as compounds with a molecular weight as high as  $10^7$  easily pass through even the smallest pores (0.1  $\mu$ m) (ref. 6). It seems more reasonable to correlate tubule induction to the differences in the penetration of cytoplasmic material through the filters. Spinal cord is able to send thin filamentous processes through 0.1- $\mu$ m pores, whereas salivary mesenchyme requires a minimum

**Table 1** Tubule-forming response of metanephric mesenchyme and penetration of cytoplasmic processes from the inductor as a function of the type of the inductor tissue and the pore size of the interposed Nuclepore filter\*

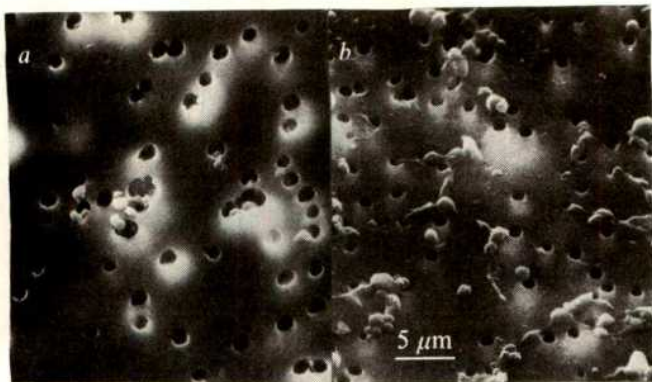
Filter Nominal pore size ( $\mu$ m)	Inductor			
	Salivary mesenchyme Tubules	Penetration	Spinal cord Tubules†	Penetration
0.1	‡		3/30	+
0.2	0/13	—	27/31	++
0.5-0.6	0/10	±	25/25	++
3.0	8/14	+	15/15	++

\*Metanephric mesenchyme and spinal cords were dissected from 11-day mouse embryos (CBA/T6T6 $\times$ BALB/c), and salivary mesenchymes from the submandibular gland of 13-d embryos. Tubule formation was evaluated by light microscopy after 72 h of cultivation<sup>12</sup>. Nuclepore filters with a thickness of  $\sim$  15  $\mu$ m and a nominal pore size of 0.2, 0.6 or 3.0  $\mu$ m (General Electric Co., Pleasanton, California, USA) were used. According to previous measurements, the largest pores are, however, 1.5-2.0  $\mu$ m in diameter<sup>6</sup>.

†Results from ref. 6.

‡Not determined.





**Fig. 1** *a*, Scanning electron micrograph of the upper surface of a Nucleopore filter with a nominal pore size of 3.0 µm. Salivary mesenchyme cultured for 48 h on the lower surface of the filter can be seen to send cytoplasmic processes through the pores. *b*, Scanning electron micrograph of the upper surface of a 0.6-µm pore size Nucleopore filter with a piece of spinal cord cultured on the lower surface for 48 h. Numerous cytoplasmic processes can be noted on the upper surface of the filter membrane. Tissues were placed on one side of the filter only. The inductor tissues (spinal cord, salivary mesenchyme) were placed below the filter and the responding metanephric mesenchyme on top of it. After 48 h of cultivation, the explants were rinsed in PBS, fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.8 for 60 min at room temperature, dehydrated in ethanol and dried by the critical point method<sup>13</sup>. Subsequently the specimens were covered with a thin layer of carbon and gold in a Balzers Micro-BA-3 evaporator and examined in a Jeol JSM-U3 scanning electron microscope (the Electron Microscope Laboratory, University of Helsinki).

pore size of 0.6 µm. Furthermore, induction of kidney tubules was never seen in transfilter situations which did not allow cytoplasmic penetration and development of contacts between inductor cells and the responding mesenchyme. The results also show that cytoplasmic penetration of filters can occur without the passage of the morphogenetic signal. The reason for this may be that contacts are either established too late, as the penetration time is a function of pore size<sup>6</sup>, or are too few. In another interactive system where the response can be quantified, the intensity of induction is a function of pore size and filter thickness<sup>15</sup>, and may correlate to the size of the contact area.

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## Haploid mouse blastocysts developed from bisected zygotes

INTEREST in haploid mouse embryos as a material for studying gene action in early development and as a potential source of haploid cell lines has stimulated a number of attempts to produce this condition experimentally. We describe here a new method of producing haploid mouse embryos by bisecting fertilised eggs. Morphologically normal haploid and haplo-diploid mosaic blastocysts were formed after insertion of such fragments to empty zonae and transfer to the oviduct.

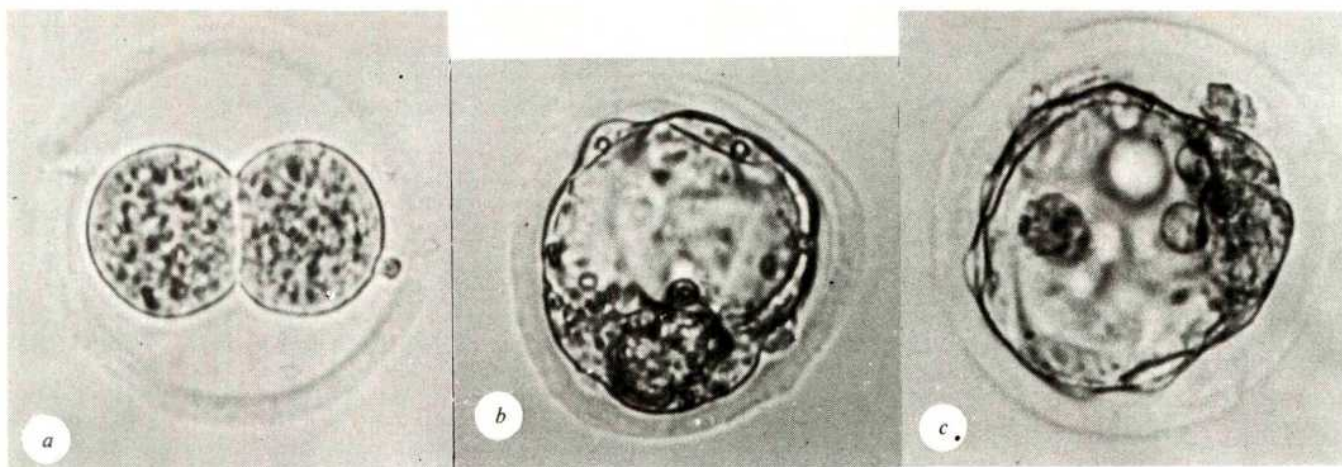
Haploid blastocysts can now be routinely obtained after parthenogenetic activation of unfertilised eggs (for review, see refs 1–3). When transferred to ectopic sites such embryos give rise in a small proportion of cases to 'growths' composed of many types of differentiated tissues, some of which may be haploid<sup>4,5</sup>. No success has yet been reported, however, in establishing haploid mammalian cell lines in culture. Haploid mouse embryos can also be obtained after microsurgical removal of one pronucleus from the fertilised egg, but for unknown reasons, very few develop into blastocysts<sup>6</sup>. Another possible method of producing haploid embryos from fertilised eggs would be to cut the pronucleate egg into halves, each containing one pronucleus. A.K.T. has recently developed such a technique (unpublished).

Pilot experiments showed, however, that early one-cell eggs subjected to harsh manipulation including microsurgery do not develop very well in culture and we decided, therefore, to test the viability of the bisected eggs in optimal conditions—that is, *in vivo*—before attempting a large scale experiment *in vitro*. As the zona pellucida is removed from the egg during the cutting procedure, the egg fragment must be inserted back into empty zonae before transfer to recipients because, up to the eight-cell stage, zona-free eggs do not survive in the oviduct<sup>7,8</sup>.

Spontaneously ovulating F<sub>1</sub>(CBA×C57BL/10) females mated with CBA-T6T6 males, and 129/J females mated with 129/J males, were killed in the early afternoon (1400–1530) on the day of the vaginal plug. The eggs were treated with hyaluronidase in phosphate-buffered saline (PBS) (200 IU ml<sup>-1</sup>) to remove cumulus cells, subjected to 0.5% Pronase to dissolve the zona pellucida<sup>9</sup>, cut into halves (A.K.T., unpublished) and cultured overnight in Whitten's medium<sup>10</sup> in liquid paraffin. Those in which both halves had divided once were transferred to PBS+0.1% bovine serum albumin and inserted microsurgically into empty zonae prepared by sucking out the contents of unfertilised eggs<sup>11,12</sup>. Blastomeres in zonae (Fig. 1a) were transferred to the oviducts of mice on day 1 of pseudopregnancy<sup>13</sup>. Three pairs of blastomeres originating from halves containing both pronuclei were also inserted into zonae and transferred to one recipient. Seventy-two hours later (day 4 of pseudopregnancy, day 5 of development of eggs) the genital tracts were flushed with PBS and the recovered embryos cultured for 3–4.5 h in medium containing Colcemid (0.2 µg ml<sup>-1</sup>) and examined in air-dried preparations<sup>14</sup>.

Table 1 summarises the results. The overall effectiveness of transplanting pairs of blastomeres into empty zonae was about 65% (range 50–100%). The recovery of transplanted eggs was, however, very low—only 16.6%. The collected embryos included six blastocysts (9%), two morulae, and three irregular embryos arrested in cleavage. These embryos together with empty zonae found in some recipients accounted for 40.3% of transplanted eggs. The majority of transplanted pairs of blastomeres most probably escaped from the zonae through the incision shortly after being transferred to the oviduct, and degenerated<sup>11</sup>.





**Fig. 1** *a*, Pair of blastomeres developed *in vitro* from a haploid egg half and transplanted into empty zona pellucida ( $\times 450$ ). *b*, 4.5-d-old gynogenetic haploid blastocyst of  $F_1(\text{CBA} \times \text{C57BL}/10)$  genotype ( $\times 600$ ). *c*, 4.5-d-old diploid blastocyst of  $F_1(\text{CBA} \times \text{C57BL}/10)$  genotype developed from an egg half which contained both pronuclei ( $\times 450$ ).

Other factors which may have been responsible for the low recovery are degeneration due to injury produced by the two successive microsurgical operations and possibly also early death of androgenetic haploids lacking an X chromosome (diploid OY embryos are believed to die during early cleavage<sup>13</sup>). The last factor could account for the loss of 25% of transplanted haploids.

In spite of the low effectiveness, these experiments show that haploid egg fragments obtained by bisecting the pronucleate egg are viable and can develop into regular blastocysts composed of both trophoblast and inner cell mass (Fig. 1*b*). The number of cells (nuclei+metaphase plates) in the six available blastocysts were as follows: 29, 32, 38, 39, 57, and 68. In three blastocysts all metaphase plates were haploid but the other three had both haploid and diploid plates ( $4 \times n + 3 \times 2n$ ;  $2 \times n + 1 \times 2n$ ;  $8 \times n + 1 \times 2n$ ) thus showing that diploidisation of some cells during cleavage which leads to  $n/2n$  mosaicism, is not peculiar to parthenogenetic haploids<sup>16,17</sup>.

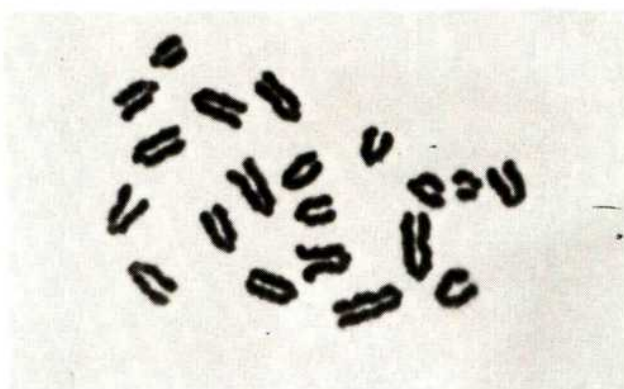
In the case of  $F_1(\text{CBA} \times \text{C57BL}/10)$  eggs fertilised by CBA-T6T6 sperms it was possible to determine the gynogenetic or androgenetic origin of embryos on the basis of the presence or absence of the T6 marker chromosome. Out of five such blastocysts four definitely had no T6 chromosome and were therefore gynogenetic. In the fifth blastocyst the presence of the marker chromosome was suspected, but because the quality of the only complete metaphase plate was not very good, the ability of androgenetic halves to develop to blastocysts remains to be confirmed. The 129/J blastocyst clearly did not contain a Y chromosome (Fig. 2) but because of the lack of karyological

markers it was not possible to determine whether it carried the genome derived from the egg or from the sperm.

The only embryo recovered after transferring three pairs of blastomeres derived from egg halves containing two pronuclei, was a regular blastocyst (Fig. 1*c*) composed of 42 cells and containing in the karyotype—according to expectations—one T6 chromosome.

In view of these encouraging results attempts have been undertaken to analyse in detail development of haploid egg

**Fig. 2** Haploid metaphase plate from a blastocyst developed from a bisected 129/J egg. There is no Y chromosome in the karyotype but because of the lack of chromosome markers the origin of the blastocyst (gynogenetic or androgenetic) cannot be determined.



**Table 1** Effectiveness of transplanting pairs of blastomeres into empty zonae

Ploidy	Genotype of eggs	No. of cleaved halves used for insertion into empty zonae	No. of cleaved halves successfully inserted and transferred to recipients	No. of transfers	Stage and no. of recovered embryos (+ empty zonae)
$n$	$\text{♀ } F_1 (\text{CBA} \times \text{C57BL}/10)$ $\times$ $\text{♂ CBA-T6T6}$	93	60	11	5 Blastocysts 2 Morulae 2 Irregular embryos arrested in cleavage 13 Empty zonae
	129/J $\times$ 129/J	9	7	2	1 Blastocyst 1 Irregular embryo arrested in cleavage 3 Empty zonae
$2n$	$\text{♀ } F_1 (\text{CBA} \times \text{C57BL}/10)$ $\times$ $\text{♂ CBA-T6T6}$	3	3	1	1 Blastocyst



fragments *in vitro* in conditions which enable the fate of sister halves from each egg to be followed.

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## Somatic cell genetic evidence for X-chromosome linkage of three enzymes in the mouse

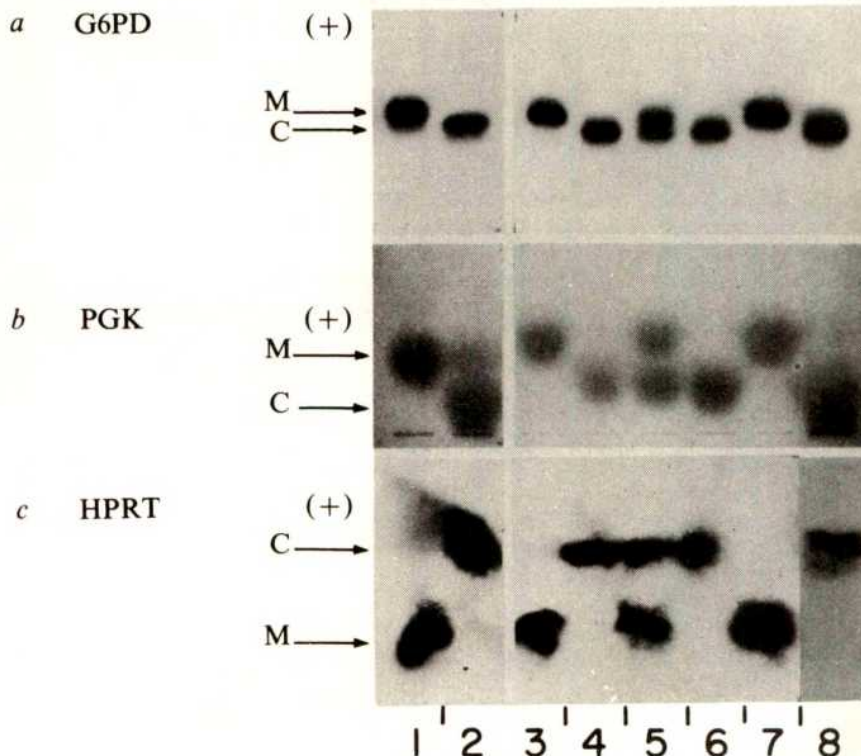
THE X linkage of loci coding for glucose-6-phosphate dehydrogenase (G6PD), hypoxanthine-phosphoribosyl transferase (HPRT), and phosphoglycerate kinase (PGK) has been determined for the mouse species *Mus musculus* and *M. caroli*. Evidence was obtained from a somatic cell genetic analysis of cultured embryonic cells of an interspecific hybrid foetus. The X-chromosomal assignment of

these loci has been determined already for several other mammalian species. This evidence was obtained from genetic analyses of electrophoretic variants<sup>1</sup> and enzyme deficiencies<sup>2,3</sup>; from the expression of these genes in viable interspecific hybrids<sup>4-6</sup>; from somatic cell genetic analyses of heterozygotes<sup>7-9</sup>, and from the expression of these loci in interspecific somatic cell hybrids<sup>10</sup>.

Evidence that these loci are X-linked in the laboratory mouse, *M. musculus*, comes from the observation that both X chromosomes are active in female germ cells and that gene dosage for these loci is doubled in oocytes of normal XX females as opposed to XO females. Activity measurements of G6PD<sup>11</sup>, HPRT<sup>12</sup>, and PGK<sup>13</sup> in oocytes has shown that oocytes from XX females contain about double the activity of each of these enzymes compared with oocytes from XO females. No genetic variants for any of these enzymes have been reported in the mouse to establish independently X linkage of these loci.

We have, however, observed that *M. musculus* and *M. caroli*, a related species from Dr J. T. Marshall in Thailand, have different electrophoretic mobilities for a number of enzymes including G6PD, HPRT and PGK. Hybrids of *M. caroli* and *M. musculus* have not been reported in the wild and they cannot be mated in laboratory conditions. Both species, however, have 40 telocentric chromosomes<sup>14</sup> and a similar reproductive cycle (V.M.C., unpublished). Interspecific hybridisation between *M. caroli* and *M. musculus* was attempted using artificial insemination of *M. musculus* females with *M. caroli* sperm (unpublished results of V.M.C. and J. Karr). No hybrid offspring were born but successful implantation and development to the foetus stage (approximately 14-16 d) was observed in three cases.

A critical feature of X-gene expression is that gene dosage is doubled in females compared with males, but the levels of gene products between the sexes is equal. The basis of this compensation between the sexes is attributable to the inactivation of one of the X chromosomes in all the somatic cells of the female<sup>15,16</sup>. This inactivation occurs early in mammalian development, possibly by the blastocyst stage. Maternally and paternally derived X chromosomes are inactivated at random so that the adult female has



**Fig. 1** Electrophoretic patterns of G6PD (a), PGK (b) and HPRT (c) in tissue and tissue culture homogenates. M represents the *musculus* forms and C represents the *caroli* forms. 1, *musculus* kidney; 2, *caroli* kidney; 3, clone B-5; 4, clone B-1; 5, cell population C; 6, clone C-6; 7, clone C-4; 8, clone A-1. The prefix for each clone indicates the mass population from which the clone was derived. Homogenates of cells from cell population A and B and mixtures of kidney homogenates demonstrated the same phenotype as cell population C in channel 5. The apparent extra PGK band in *caroli* kidney (b, 2) is a minor anodal band in the PGK phenotype that is present at high concentrations of tissue extracts. These minor components were observed in tissues of both males and females of *M. caroli*. A similar minor component is seen in tissue extracts of *M. musculus*, but at even higher concentrations. These minor components were not observed in cell culture extracts.

**Table 1** Distribution of gene markers in clones of cultured cells derived from a *M. musculus* × *M. caroli* foetus

Population	Total clones	Six linked enzymes			NP	Autosomal enzymes	
		G6PD	PGK	HPRT		GPI	GOT-1
A	1	MC	MC	MC	H	H	H
		C	C	C	H	H	H
B	2	MC	MC	MC	H	H	H
		C	C	C	H	H	H
C	5	M	M	M	H	H	H
	5	MC	MC	MC	H	H	H
	3	C	C	C	H	H	H
	3	M	M	M	H	H	H
	16						

For each clone, all enzymes were analysed on the same cell homogenate. M denotes *musculus* origin; C denotes *caroli* origin; MC denotes both *musculus* and *caroli* contributions; and H denotes a hybrid phenotype with parental and heteropolymeric electrophoretic forms.

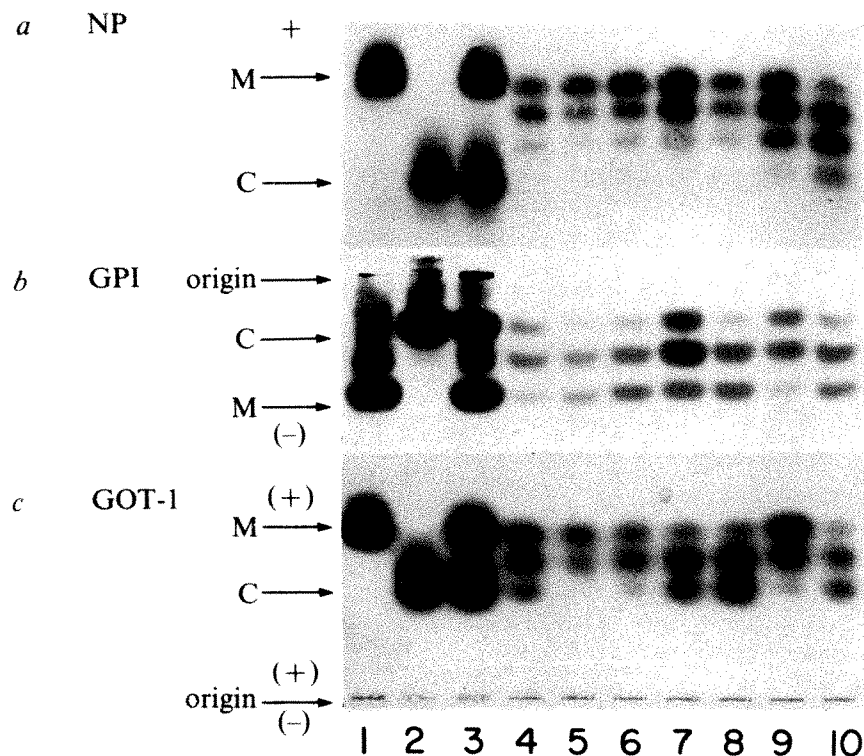
two populations of cells; one expressing the maternal and the other the paternal X.

If G6PD, HPRT and PGK are X-linked in *Mus* species, we reasoned that both *musculus* and *caroli* forms would be expressed in a hybrid female foetus and only the *musculus* form would be expressed in a male foetus. We therefore expected that a primary cell culture of hybrid cells would have both *caroli* and *musculus* electrophoretic forms if the foetus were female, or only *musculus* forms in the case of a male. The resulting cell lines from the hybrid foetus had both *caroli* and *musculus* electrophoretic forms of G6PD, HPRT and PGK and were presumed to be female. In the case of a female we further reasoned, from the Lyon hypothesis, that clones derived from single cells of the culture should uniformly express either the *caroli* or *musculus* electrophoretic forms of all three enzymes if these loci are X-linked. Hybrid foetal electrophoretic phenotypes and cloning of cultured foetal cells showed that these enzymes segregated as predicted for X-linked loci in *M. caroli* and *M. musculus*.

Foetuses were dissected from the uterus 16 d after arti-

cial insemination. Two were homogenised in equal volumes of distilled water and assayed for electrophoretic phenotypes. The third foetus was used to establish a primary cell culture: the 16-d embryo was minced in a solution of proteolytic enzymes (Viocase, Gibco) and Eagle's medium without serum, and divided into three parts, each of which were plated separately in Dulbecco's modified Eagle's medium (DMEM) (high glucose formula) with antibiotics and 10% foetal calf serum (Gibco). The cells were maintained in an atmosphere of 5–7% CO<sub>2</sub>. A monolayer of proliferating cells was obtained from each of the three original cell suspensions and designated primary cell populations A, B and C. Each cell population was maintained separately in the above medium and single cells were cloned by the procedure of Puck *et al.*<sup>17</sup>. Cell populations and clones were grown to confluency, collected for enzyme identification, and homogenised at a concentration of 7 × 10<sup>7</sup> cells per ml 0.5 M Tris at pH 7.5 (ref. 18). G6PD (EC 1.1.1.49) PGK (EC 2.7.2.3) and HPRT (EC 2.4.2.8) were analysed by starch gel electrophoresis<sup>10,19</sup>.

Electrophoretic phenotypes of *M. caroli* and *M. musculus*



**Fig. 2** Electrophoretic patterns of: NP (a), GPI (b) and GOT-1 (c) in tissue and tissue culture homogenates. M represents *musculus* forms and C represents *caroli* forms. 1, *musculus* kidney; 2, *caroli* kidney; 3, 1:1 mixture of *musculus* and *caroli* homogenates; 4, cell population B; 5, clone B-5; 6, clone B-1; 7, cell population C; 8, clone C-6; 9, clone C-4; 10, clone A-1. In cell culture extracts, the two heteropolymers migrating between the parental forms of NP indicate that NP is a trimeric enzyme<sup>21</sup>. The single heteropolymer observed for GPI and GOT-1 in cell culture extracts indicates that these enzymes are dimers. All cell culture extracts of hybrid populations demonstrate both parental and heteropolymer forms, indicating that both parental genes for each enzyme are active in each cell. The *musculus* and *caroli* tissue GPI phenotypes possess a major band and several anodally migrating minor bands at the high concentrations used for tissue extracts (b, 1 and 2). These minor bands were not observed in cell culture extracts. GPI is a dimeric enzyme and a hybrid enzyme is observed in heterozygotes for GPI electrophoretic variants in the mouse<sup>22</sup>. The band intermediate to the two parental forms in the 1:1 mixture (b, 3) is a *musculus* minor component and not a heteropolymer. A hybrid GPI enzyme, compared with the parental forms, is more intense as demonstrated by the relative intensities of the parental and hybrid enzymes in the cell culture samples. The variability between clones with regard to intensity of enzyme bands probably represents an increase in the number of

gene copies since chromosome numbers increased as a result of the foetal cells becoming established as cell culture lines. Chromosomes were examined on two hybrid clones from cell population B, one expressing *musculus* and the other *caroli* X-linked enzymes. They had mean chromosome numbers of 69 and 63, respectively, and only telocentric chromosomes were observed.



kidney HPRT, PGK and G6PD are shown in Fig. 1. The major band of activity for each enzyme has a different mobility for each species. One hybrid foetus demonstrated both *musculus* and *caroli* forms and was presumably a female, and one foetus demonstrated only *musculus* forms and was presumably male. These findings are consistent with X linkage. In each of the three primary cell populations, the electrophoretic patterns consisted of both *musculus* and *caroli* forms for each enzyme. The electrophoretic phenotypes of the three enzymes for cell population B is shown in Fig. 1 (channel 5). Sixteen clones derived from single cells were examined. The clones were established from all three populations soon after the establishment of the primary cell culture. Eight of the clones coordinately expressed only *musculus* electrophoretic types for PGK, HPRT and G6PD and eight coordinately expressed only *caroli* forms (Fig. 1 and Table 1). These findings support the prediction that the primary cell populations are mixtures of cells with either an active *musculus* X chromosome or an active *caroli* X chromosome.

The expression of three autosomal enzymes which differ in electrophoretic phenotype between the two species was tested to demonstrate that the foetuses, the cells of the primary cell populations and the clones were true hybrids with functional *musculus* and *caroli* genes in the same cell. Cytoplasmic glutamic oxaloacetic transaminase (GOT-1) (ref. 20), nucleoside phosphorylase (NP) (ref. 21), and glucosephosphate isomerase (GPI) (ref. 22) are multimeric enzymes which differ electrophoretically between *musculus* and *caroli* (Fig. 2). Each of these enzymes is coded by an autosomal locus as determined by genetic tests in *M. musculus* or man. Since these enzymes are multimeric, it was expected that electrophoretic phenotypes demonstrating both parental forms and intermediate hybrid forms would be observed if both parental genes were expressed in the same cell. Hybrid electrophoretic patterns consistent with the formation of intermediate heteropolymers by random combination of *musculus* and *caroli* polypeptide subunits in individual cells were demonstrated in the foetuses, the three cell populations and the clones (Fig. 2 and Table 1). Mixtures of *musculus* and *caroli* kidney extracts produced additive electrophoretic patterns without intermediate hybrid enzymes (Fig. 2). All 16 clones demonstrated both parental and heteropolymer bands for the autosomal enzymes (Table 1). Thus, both *caroli* and *musculus* genes were active in the original cell which formed the clones and remained active through successive cell generations. On the other hand, each clone showed only the *caroli* or the *musculus* phenotype for PGK, G6PD and HPRT. We conclude that these results are consistent with the predictions of the Lyon hypothesis that there are two populations of cells, that only one X chromosome is active in a cell, and that the same X remains active in successive cell generations. In retrospect, two populations of cells were indicated in one foetus and in the cells of the hybrid foetus used to make the primary culture by the two banded G6PD electrophoretic pattern. Because G6PD is a dimer, one expects a three banded electrophoretic phenotype like GPI or GOT-1 (Fig. 2) if both chromosomes are active in a single cell. Only both parental forms were observed, however, (Fig. 1). The electrophoretic findings of hybrid enzymes for NP, GPI and GOT-1 demonstrate that both *musculus* and *caroli* genes function in the same cell and that the foetuses were interspecific hybrids.

The strategy of analysing clones from single cells of heterozygous females has demonstrated the X linkage of HPRT, G6PD and PGK in man<sup>7-9</sup> and G6PD in mules<sup>5</sup>. In each instance only one of the two possible parental forms demonstrated by a heterozygous female was expressed. Such somatic cell genetic analyses have been confirmed in man by family studies using genetic variants of each enzyme. The segregation of PGK, G6PD and

HPRT in clones of somatic cells from an interspecific hybrid foetus and foetal extract phenotypes demonstrates an X-chromosome assignment of these enzyme loci in mice as reported from a comparison of enzyme activities in XO and XX oocytes<sup>11-13</sup>. These conclusions also agree with Ohno's hypothesis of conservation of the X chromosome among mammalian species<sup>24</sup>.

The relative amounts of *musculus* and *caroli* PGK, G6PD and HPRT expressed in the three primary cell populations were approximately equal. This suggests that the proportion of the cells with active *musculus* or *caroli* X chromosomes may have been equal in the hybrid foetus. Thus, there is no evidence that either parental X chromosome was preferentially inactivated during development or that cell selection acts to distort the proportions of the two cell populations. This finding is similar to the observation of X-chromosome expression in interspecific hybrids such as the mule<sup>4,5</sup> and in crosses of European hare species<sup>8</sup>, indicating that the genetic mechanism for X-chromosome inactivation is also conserved in the speciation of eutherian mammals.

In conclusion, although we have not yet obtained hybrid progeny developing to term, the combination of (1) multiple X-linked biochemical differences, (2) random inactivation of *caroli* and *musculus* X chromosomes in hybrid embryos, and (3) the occurrence of some hybrid foetuses which seem grossly normal at 14-16 d of development, suggests that the hybrid combination of *M. musculus* and *M. caroli* is a promising experimental system for studying X inactivation in mammalian development.

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## Imperfect complementation in human-hamster somatic cell hybrids

SOMATIC cell hybridisation can reveal complementation between genetic markers<sup>1</sup>, and interspecific hybridisation provides a means to study the mechanisms involved in



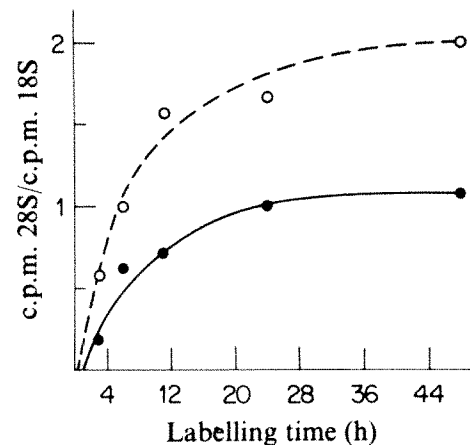
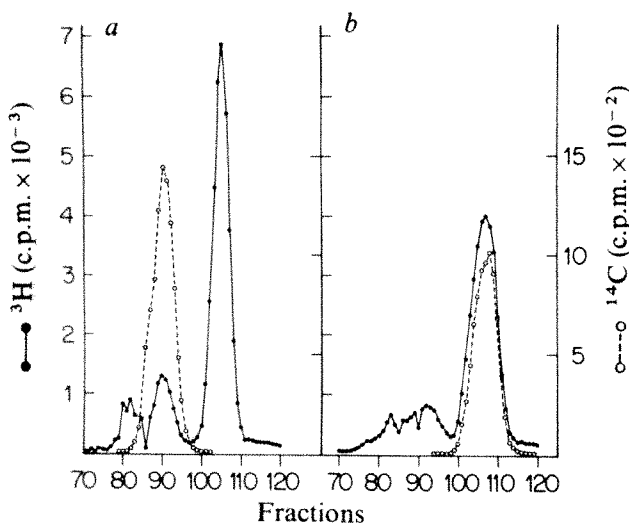
complementation. When the phenotypes of both parental cells are distinguishable at the molecular level, two complementation mechanisms can be recognised<sup>2</sup>. When the allele contributed by the wild type parental cell overcomes the block in the biochemical pathway characteristic of the mutant parent, products characteristic of both species should be present in the hybrid. Alternatively, if either allele acts exclusively on its own pathway, that is the wild-type allele cannot act in *trans*, the blockage due to the mutation remains and only the wild-type product would be expected to be synthesised in the hybrid.

We have reported<sup>2</sup> complementation of a defect in the production of ribosomal RNA (rRNA) in interspecific cell hybrids. Mutant *ts422E* is a temperature sensitive (*ts*) variant of the Syrian hamster line BHK 21/13 (ref. 3). At the non-permissive temperature (39 °C) the cleavage of nucleolar 32S RNA to produce mature ribosomal 28S RNA is blocked. rRNA processing and production are otherwise normal at 33 °C<sup>4</sup>. When *ts422E* is hybridised with mouse cells the resulting hybrids produce both hamster and mouse 28S RNA at the non-permissive temperature<sup>2</sup>, indicating that in this case complementation occurs by the first of the two possible mechanisms. In an attempt to characterise further the nature of the complementation between interspecific rRNA maturation genes, we hybridised *ts422E* with human cells. We found that complementation of the defect borne by the *ts422E* hamster cell mutant is only partially achieved in hybrids with human cells. In this case the same mechanism of complementation operates as in *ts422E*-mouse hybrids, but only to a partial extent.

*ts422E* cells were fused with LW SV40-transformed human cells deficient in hypoxanthine phosphoribosyltransferase<sup>5</sup> in the presence of  $\beta$ -propiolactone-inactivated Sendai virus at pH 8.0 (ref. 6). Hybrid cells were selected in HAT medium<sup>7</sup> at 39 °C. Hybrid colonies were obtained at a frequency of about  $1 \times 10^{-5}$  of the total cells plated in selective conditions; they were isolated and propagated in HAT medium at 39 °C for one passage. Hybrid lines were propagated subsequently in normal medium at 39 °C unless otherwise stated.

We used populations of hybrids obtained on two plates. The populations grew poorly at 39 °C, but in an essentially

**Fig. 1** Hybrid population F3 growing at 39 °C was labelled for 13 h with <sup>3</sup>H-uridine (6  $\mu$ Ci, 2  $\mu$ g ml<sup>-1</sup>). Cells were washed, the cytoplasmic fraction prepared and the 28S rRNA isolated after centrifugation in sucrose gradients as described before<sup>2</sup>. The RNAs were analysed by polyacrylamide gel electrophoresis. Electrophoreses were run for 24 h at 5 mA per gel. Gels were sliced and counted as described in ref. 2. Samples were run with <sup>14</sup>C-labelled markers prepared from BHK or HeLa cells. *a*: ●, 28S <sup>3</sup>H-RNA from population F3; ○, HeLa <sup>14</sup>C-28S RNA. *b*: ●, 28S <sup>3</sup>H-RNA from population F3; ○, BHK <sup>14</sup>C-28S RNA.



**Fig. 2** Plates containing hybrid 53-99 C6 were incubated for 5 d at 33 or 39 °C and labelled for different times with <sup>3</sup>H-uridine (16, 8 or 4  $\mu$ Ci; 2, 4, or 8  $\mu$ g ml<sup>-1</sup>). At the end of the pulse cells were fractionated as described in caption for Fig. 1. RNA from cytoplasmic fractions was purified by double phenol extraction and precipitated with 2 volumes of 95% ethanol at -20 °C. RNA was subjected to polyacrylamide gel electrophoresis in the conditions described in Fig. 1. Electrophoreses were run for 4.5 h. 28S and 18S rRNA peaks were integrated in each case and the ratio of c.p.m. in the 28S peak relative to the amount of radioactivity in the 18S peak (28S:18S ratio) was determined. ●, Data corresponding to 39 °C; ○, data corresponding to 33 °C.

normal manner at 33 °C. Giemsa banding by a modification of Seabright's method<sup>8,9</sup> showed that the hybrid F3 contained human chromosomes 3, 14, 15, 17 and 20 and two abnormal chromosomes, probably of human origin. The hybrid F31 had human chromosome 3 and one abnormal human chromosome. It is also expressed human mannose phosphate isomerase (MPI). All hybrid cells contained the entire complement of hamster chromosomes.

The 28S rRNA made by the populations F3 and F31 at 39 °C was analysed in 2.6% polyacrylamide gels, where hamster 28S RNA migrates faster than human 28S RNA<sup>10</sup>, as described in Fig. 1. F3 synthesised mostly hamster 28S rRNA but some human rRNA was also present; results were similar for population F31 (Fig. 1).

For subsequent studies we used a clone of the F3 population. The clone 53-99 C6 also grew poorly at 39 °C. It contained human chromosome 3 in five and human chromosome 17 in two of 20 metaphases analysed. These hybrid cells also expressed human MPI activity, but not human pyruvate kinase 3 or hexosaminidase A, suggesting that they contained only a portion of human chromosome 15 (refs 11 and 12). Table 1 compares the doubling times of the 55-99 C6 hybrid at 33 and 39 °C with those of wild-type BHK, the hamster parent *ts422E* and one of the mouse-*ts422E* hybrids, hyC7. Growth in all *ts*<sup>+</sup> cells was faster at 39 than at 33 °C, with the exception of the *ts422E*-human hybrid, suggesting inefficient, although positive, complementation.

To determine whether the slower growth at 39 °C was a consequence of reduced production of 28S rRNA, the kinetics of labelling of rRNA were studied at this temperature. The amount of radioactivity in 18S rRNA was used as a reference as 18S rRNA is normally synthesised in the parental *ts* mutant at either temperature<sup>3</sup>. 53-99 C6 cells were labelled for different times, and cytoplasmic RNA was isolated as before and analysed in polyacrylamide gels<sup>4</sup> (Fig. 2). At 33 °C the ratio of label in 28S RNA to that in 18S RNA increased, to reach a steady state at ~1.9 after 24 h. At 39 °C the steady state was reached more slowly and the ratio never exceeded 1.2. Similar results were obtained with the original F3 and F31 hybrid populations. Thus, the slow growth of the hybrid at 39 °C was probably due to impaired production of 28S rRNA.

At either temperature, hybrid 53-99 C6 synthesised 28S rRNA of hamster origin only (Fig. 3; results obtained at

**Table 1** Doubling times of several cell lines at 33 and 39 °C (h)

	33 °C	39 °C
53-99 C6 ( <i>ts422E</i> -human hybrid)	29	37
C7 ( <i>ts422E</i> -mouse hybrid)	23	18
<i>ts422E</i>	18	∞
wild-type BHK21	16	12

Plates (60 mm) were seeded with  $5 \times 10^4$  cells per plate and incubated at 33 or 39 °C. Every 24 h duplicate plates from each temperature were trypsinised and the cells counted with a Coulter counter.

39 °C were identical). This suggests that the human nucleolar organisers<sup>13,14</sup> have been segregated out in this hybrid, although alternative hypotheses cannot be ruled out.

To determine whether the reduced production of 28S RNA in the hybrids at 39 °C was the expression of the defect of the *ts422E* parent, corrected only to some extent, we analysed the pattern of nucleolar RNA processing. *ts422E* cannot produce 28S RNA because of a block in the processing of the 32S RNA precursor, which, after production is mostly degraded, although a portion of it accumulates to levels 8–10 times greater than in wild-type cells<sup>4</sup>.

Table 2 summarises the distribution of label in nucleolar 32S RNA and cytoplasmic 28S rRNA in hybrid 53-99 C6 after an 11-h pulse at 33 or 39 °C. Data for BHK and *ts422E* at 39 °C are also included. As 32S (28S) and 18S RNAs are derived in a 1:1 molar ratio from a common 45S RNA precursor<sup>13,14</sup>, the 32S:18S or 28S:18S ratios enable us to normalise the differences due to causes other than the mutation itself, such as differences in uridine pools or uptake in different cell lines. At 39 °C the 28S:18S ratio in the hybrid is intermediate between the values for wild-type and mutant cells; the same was true for the 32S:28S ratio. The 32S:18S ratio showed clearly that much more 32S RNA accumulated in the hybrid at 39 °C than in wild-type BHK, the degree of accumulation being similar to that in *ts422E* cells. The accumulation of 32S RNA in the hybrid, however, was greater than expected at 33 °C, perhaps because the steady state had not yet been reached (Fig. 2). Thus rRNA precursors are processed in *ts422E*-human hybrids at 39 °C in a pattern similar to that in the *ts422E* mutant, with comparable accumulation (and degradation) of the 32S RNA precursor. This indicates that the slow growth of the hybrids at 39 °C is due to imperfect complementation (albeit compatible with survival) of the *ts422E* defect.

Although we wished to map the gene(s) responsible for processing of rRNA precursors on human chromosomes all hybrids examined contained chromosomal rearrangements and it was impossible to assign the gene(s) involved in the complementation of the *ts422E* defect.

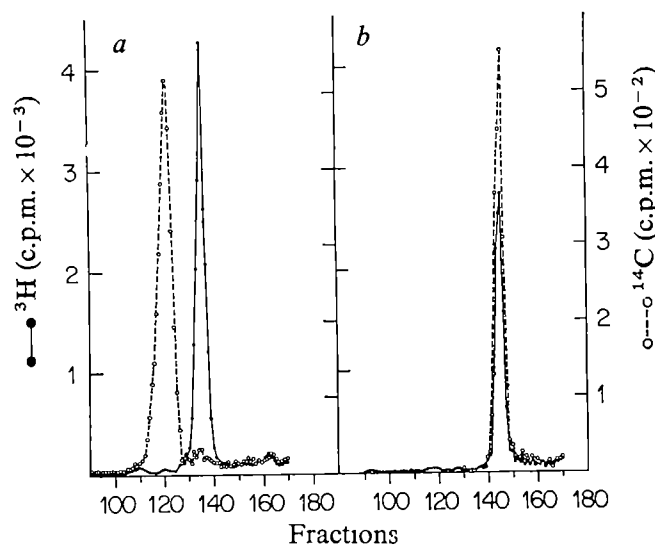
The imperfect complementation can be explained by

assuming that, although the rRNA processing machinery can act on RNAs from related species as efficiently as on RNA from the same species<sup>2</sup>, in phylogenetically distant species such as man and hamster the sites of recognition of rRNA diverged during evolution. This could result in inefficient recognition or binding of accessory preribosomal proteins to the rRNA precursors<sup>15</sup>. In this respect it should be remembered that 53-99 C6 hybrid was selected and isolated at the temperature restrictive for the parental mutant. It is thus reasonable to assume that the level of complementation found in the hybrid is the best the population could attain under the pressures imposed.

Why did no human 28S RNA appear at either temperature in the hybrid, resembling the situation found<sup>16</sup> in human-mouse hybrids? Among the hypotheses considered<sup>18</sup> the most probable is the repression of human rRNA genes due to the presence of mouse nucleolar organisers. The situations in the human-hamster hybrid 53-99 C6 could be similar, but the presence of some human 28S rRNA in hybrid populations F3 and F31 suggests that this is not compulsory. It is possible, in our case, that the disappearance of human rRNA during serial culture was due to loss of human rDNA genes.

It seems unlikely that poor complementation is due to insufficient human gene products in the hybrid. The selection of hybrid lines at 39 °C, and in particular their poor

**Fig. 3** Plates containing hybrid 53-99 C6 were incubated at 33 °C for 6 d and labelled for 11 h with <sup>3</sup>H-uridine (4.4 μCi, 2 μg ml<sup>-1</sup>). 28S rRNAs were prepared and analysed by polyacrylamide gel electrophoresis in the conditions given in Fig. 1. Electrophoreses were run for 24 h at 5 mA per gel. Samples were run with <sup>14</sup>C-labelled markers from BHK or HeLa cells. *a*, 28S rRNA (<sup>3</sup>H) from hybrid 53-99 C6 (●); HeLa 28S <sup>14</sup>C RNA (○); *b*, 28S rRNA (<sup>3</sup>H) from hybrid 53-99 C6 (●); BHK 28S <sup>14</sup>C RNA (○).

**Table 2** Distribution of radioactivity in different species of rRNA in *ts422E*-human hybrids

Cell line (temperature)	32S	28S	18S	Ratio 32S:18S	Ratio 32S:28S	Ratio 28S:18S
53-99 C6 (39 °C)	$4.4 \times 10^4$	$2.2 \times 10^5$	$3.0 \times 10^5$	0.15	0.20	0.72
53-99 C6 (33 °C)	$2.8 \times 10^4$	$2.8 \times 10^5$	$1.8 \times 10^5$	0.16	0.10	1.57
<i>ts422E</i> (39 °C)	$9.1 \times 10^3$	$1.2 \times 10^4$	$6.1 \times 10^4$	0.15	0.76	0.20
Wild-type BHK21 (39 °C)	$3.2 \times 10^3$	$3.6 \times 10^4$	$1.6 \times 10^4$	0.02	0.01	2.25

Cells were labelled for 11 h with <sup>3</sup>H-uridine (4 μCi, 2 μg ml<sup>-1</sup>) and fractionated as explained in Fig. 1. Nucleolar fractions were prepared as previously described<sup>4</sup>, and nucleolar RNA was analysed by polyacrylamide gel electrophoresis in the conditions given in Fig. 1. Nucleolar RNA electrophoreses were run for 9 h. Cytoplasmic RNAs were analysed as in Fig. 2. The data are expressed as c.p.m. per 10<sup>6</sup> cells.

growth at 39 °C, should have strongly favoured cells which had not lost the gene(s) complementing the *ts422E* defect.

It is difficult, however, to rule out the possibility that human chromosomes are lost continuously in the hybrid populations at 39 °C. Thus each population of hybrid cells, at any given moment, could be a mosaic of cells which have and have not retained the human gene(s) required for normal rRNA maturation and cell growth at the non-permissive temperature. Since data on rRNA production show that about 80% of the cells would have to be mutant-like to produce the observed results, this hypothesis implies that at every cell division the hybrids yield a high proportion of cells behaving as the parental *ts* mutant, and thus unable to grow at 39 °C. Such a mechanism would cause the rate of growth of the cell population to appear linear, rather than exponential, but the rate of growth of the hybrids at 39 °C was exponential (Table 1). We therefore believe this explanation unlikely.

Our data suggest that the human wild-type allele is dominant over the *ts422E* mutation, as it can process hamster rRNA precursors; this processing is, however, inefficient probably due to evolutionary divergence of the rRNAs of the two species.

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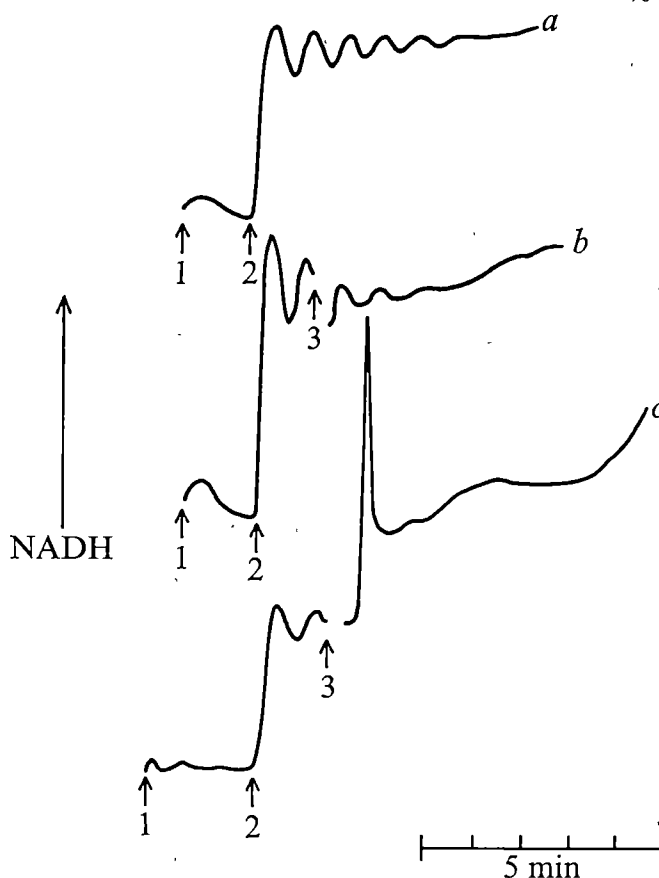
## Cell density dependence of oscillatory metabolism

THERE is increasing evidence<sup>1-3</sup> that the behaviour of cell populations is determined not only by the behaviour of the individual cells, but also by their mutual interactions. One influence on the strength of these interactions may be cellular proximity which, in turn, is a function of cell density. For example, myoblasts fuse only when they reach a critical cell density<sup>4</sup>. The oscillatory glycolytic metabolism of the yeast *Saccharomyces carlsbergensis* has provided a convenient system for the exploration of several biochemical phenomena<sup>5</sup>. For example, whereas yeast cells are generally considered to be metabolically non-interacting, a coupling process of unknown character causes metabolic synchronisation of all cells to a common oscillatory fre-

quency and phase<sup>6</sup>. A suspension of metabolically oscillating yeast therefore behaves as a population of strongly interacting units. Because continuous monitoring of amplitude and phase by fluorometry provides a constant assay of metabolic dynamics, any perturbation of these dynamics is easily recognised as a deviation from the predictable waveform. We describe here modifications in the metabolic behaviour of intact cell suspensions achieved by manipulating the strength of their interaction through changes in cell density.

*S. carlsbergensis* was grown, collected and assayed for oscillatory metabolism as described before<sup>7</sup>. The results of one experiment are shown in Fig. 1; Fig. 1a records, for a period of 8 min in an oxygenated 0.0125% (w/w) yeast suspension, the total culture fluorescence, which correlates with the intracellular concentration of NADH. Addition of 100 mM glucose at arrow 1 caused a brief surge of intermediates through glycolysis as shown by the increase in NADH concentration preceding the aerobic steady state. On addition of 1 mM KCN to block respiration, (arrow 2) a marked increase in NADH concentration initiated a damped oscillatory approach to the metabolically anaerobic steady state. The second tracing (Fig. 1b) is similar except that the initial density of the cell suspension was 4% (320 times that in the first experiment). At arrow 3 buffer was added and caused a rapid change of suspension density to 2.66%. After a short lag during which the sensitivity of the fluorometer was increased to compensate for the decrease in cell density, metabolic oscillations continued. When this experiment was repeated, but with an initial cell density of 0.0125% (Fig. 1c) rapid density reduction (arrow 3) abolished further detectable oscillatory expression. The

Fig. 1 a, Net fluorescence (NADH concentration) is plotted against time in a 0.0125% (mg wet weight per ml) yeast suspension. Arrow 1 indicates addition of glucose (100 mM final concentration) to the oxygenated culture and arrow 2 addition of KCN (1 mM final concentration). b, Same as a but initial cell density is 4% and arrow 3 indicates addition of 1 ml of buffer to the 2 ml of culture. c, Same as b but initial density is 0.0125%.



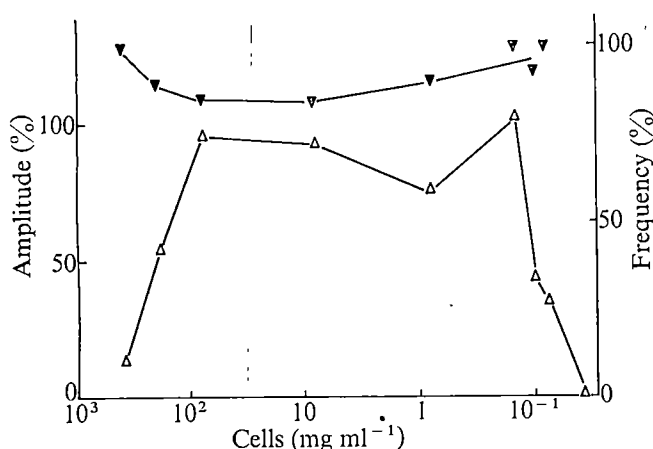


Fig. 2 Normalised amplitude and frequency are plotted against culture density (see text). 100% amplitude corresponds to 100  $\mu$ M NADH and the normalisation standard used was a 400 mg ml<sup>-1</sup> suspension. 100% frequency corresponds to 1/38 s<sup>-1</sup>. ▼, Normalised frequency; △, normalised amplitude.

sharply peaked transient following density change was a constant characteristic only of oscillatory abolition, and was never shown by suspensions which continued to oscillate. We found that, varying with time of collection, some critical initial cell density could always be found below which no oscillatory metabolism was detected after rapid reduction of cell density.

Figure 2 plots amplitude and frequency against cell density in one such batch. The fluorometer was calibrated by adding known concentrations of NADH to non-metabolising cell suspensions of various densities. Suspensions of equivalent densities then were run as in Fig. 1a. Amplitude was assayed by measuring the height of the second peak from a line connecting the first and third troughs. This distance remained constant, within  $\pm 5\%$ , in multiple identical experiments. If cell density did not affect the dynamics of each cell, a doubling in cell concentration would be expected to produce a doubling in recorded amplitude. Thus, taking the change in NADH concentration of the second peak in a 400 mg ml<sup>-1</sup> suspension as a normalisation standard, the height in an 800 mg ml<sup>-1</sup> suspension was divided by 2 and that in a 200 mg ml<sup>-1</sup> suspension by 0.5 to correct the raw data for expected density-induced changes in recorded amplitude. We assumed that identical normalised amplitudes should result if no change in dynamics resulted from cell density differences. In Fig. 2 normalised amplitude is expressed as a percentage of the largest second peak amplitude observed in any experiment in the series, and frequency is similarly expressed. Frequency changes little within the range of cell densities tested but amplitude reaches a broad maximum at intermediate densities. This agrees with the finding (Fig. 1) that low cell densities cause a loss of oscillatory metabolic expression. It also suggests that high cell densities inhibit oscillatory expression.

Two explanations for these findings seem possible. Either each cell is affected by cell density and loses its oscillatory capacity, or the population loses synchrony. Measurements made on the total suspension cannot distinguish between these two possibilities, although asynchrony is much harder to rationalise at high densities, when tight coupling might be assumed, than at low densities. Microspectrophotometric measurements<sup>8</sup> showed previously that individual cells, when oscillating, display both the frequency and waveforms similar to those expressed by the total cell suspension. Although single-cell measurements were not designed to quantify density dependence, there was some indication that a minimal density was necessary to observe single-cell oscillations. These data support the possibility that the inhibition of metabolic oscillations observed at low densities

is not caused by population desynchronisation but by alterations in the stability properties of each cell's metabolic dynamics; that is the steady state of each cell changes from an oscillatory to a non-oscillatory metabolism.

Although the mechanism by which cells synchronise their metabolism is unknown, one might expect its efficiency and characteristics to be a function of cell density and thus underlie the phenomena reported here. This suggests that short range, intercellular communication mechanisms strongly influence not only the quantitative, but also the qualitative metabolic behaviour of cells, such as oscillatory and non-oscillatory behaviour. Furthermore, metabolic synchronisation and density dependence lead to a situation in which the behaviour of the total cell population could be quite unrelated to the sum of the behaviours displayed by individual cells in isolation: that is, biochemical synergism occurs.

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## Carcinogenic nitrosamides and cell cultures

THE acylnitrosamides ethylnitrosourea (ENU) and methyl-nitrosourea (MNU) are potent carcinogens in a variety of animals. Rats, especially, provide a useful experimental system since a single dose administered at the perinatal age can be tumorigenic and in certain strains the nitrosamides in these conditions are relatively site specific in that they produce a high proportion of neural tumours<sup>1–6</sup>. Although these tumours rarely become clinically apparent before six months, the short term effects of nitrosamides have been studied both *in vitro* and *in vivo* in an attempt to elucidate the oncogenic mechanism. Thus histological examination of tissues from rats recently treated with nitrosamides has revealed a toxic effect on dividing cells<sup>7</sup> while the use of cell cultures has shown an effect on DNA synthesis and the cell cycle<sup>8</sup>. It has been assumed that both the carcinogenicity and the short term effects occur as a result of the alkylation of macromolecules, in particular DNA<sup>9</sup>. Earlier reports concerned the nature of the chemically modified bases<sup>10,11</sup> whereas more recently the turnover of such bases has become the focus of attention<sup>12,13</sup>. We present evidence that the short term effects can be explained by an alternative mechanism to the alkylation of cellular macromolecules; cyanate ions are shown to be a product of MNU and ENU breakdown and it is the cyanate ion which is responsible for short term effects and cytotoxicity.

Cell cultures were derived from the neural tissue of AS rat fetuses on day 18 of gestation, and during studies into the effect of dose and exposure time, chemical breakdown of nitrosamides was followed spectrophotometrically since they are known to decompose rapidly in physiological conditions<sup>14</sup>. Figure 1 shows the rate of decomposition in cell culture conditions and the effect of exposure time on cell survival. Since the nitrosamides decay rapidly, yet long



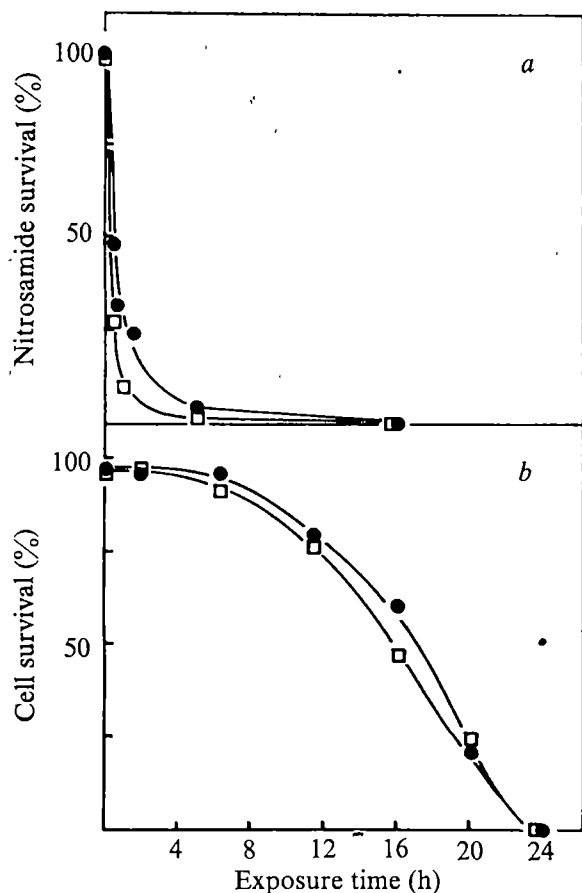


Fig. 1 *a*, MNU (□) or ENU (●) were taken up into warm ethanol at 40 mg ml<sup>-1</sup> and immediately added to Ham's F10 growth medium containing 10% FCS to give a final concentration of 1 mM. At intervals, aliquots of nitrosamide-containing medium were taken and difference-spectra obtained to assess the rate of decomposition. MNU has a peak at 380 nm and ENU at 375 nm. *b*, Primary cultures were set up from the neural tissue of AS rat fetuses on day 18 of gestation. 0.05% trypsin in phosphate-buffered saline (PBS) was used for 15 min to disperse the tissue after sterily removed brains had been very finely minced with scissors. The suspension obtained from one foetal brain was added to F10 medium with 10% serum and then plated out into three 90-mm Petri dishes. In one week such dishes usually achieved confluence. Cells were used before the third passage when a number of different cell types were still present. MNU (□) or ENU (●) were added to culture medium as in *a* to give a final concentration of 1 mM and this medium was immediately added to 50 mm plates of cells. At intervals, duplicate plates were washed twice and then incubated with untreated growth medium. At 30 h, all plates were viewed under an inverted microscope with phase-contrast optics. Healthy and lysed cells were easily distinguishable; > 200 cells were counted per plate and cell survival expressed as a percentage.

exposure times bring about cell death, a breakdown product is likely to be responsible for such cytotoxicity. This was confirmed by incubating either nitrosamide at 37 °C in growth medium for 3 d before adding the medium to cell cultures when it was found to be as toxic as medium containing freshly-prepared nitrosamide. To identify the breakdown-product, <sup>14</sup>C-methylnitrosourea and methylnitroso-<sup>14</sup>C-urea were synthesised. Paper chromatography with a solvent system of ethyl acetate-ethanol-water (80:15:5) was used to monitor breakdown of the radiolabelled nitrosamide. When methylnitroso-<sup>14</sup>C-urea decomposed in growth medium, the *R<sub>f</sub>* of the isotope changed from 1.0 to 0.26; this product was not urea or methylurea but when K-<sup>14</sup>CNO was applied, the <sup>14</sup>C cochromatographed with the breakdown-product. When <sup>14</sup>C-methylnitrosourea decomposed the isotope was lost from the *R<sub>f</sub>* 1.0 position, but did not appear elsewhere; this suggested a volatile breakdown product and it was shown by co-distillation with unlabelled material to be methanol. It was thus demonstrated that

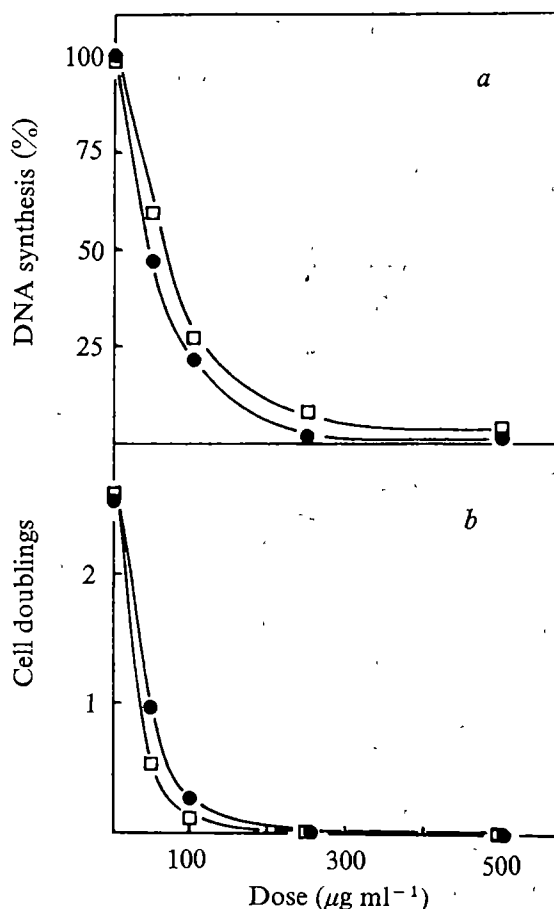
>97% of MNU breaks down in culture conditions, to yield methanol, cyanate ions and gaseous nitrogen.

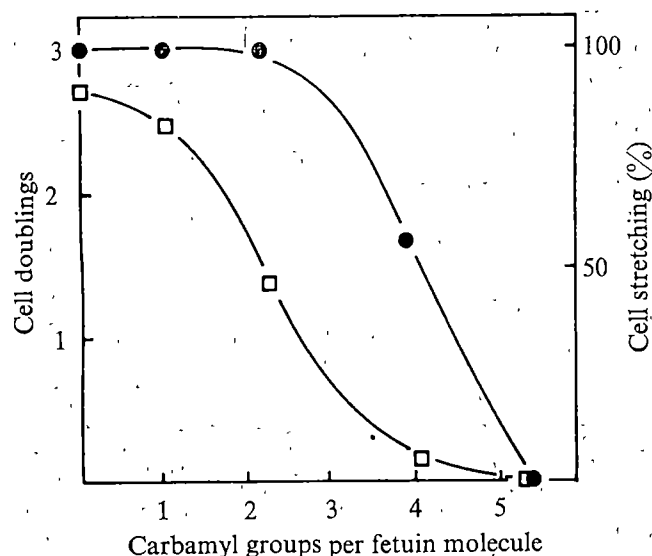
Cyanate ions were the only possible cytotoxic agent and indeed the cell death induced by nitrosamides is mimicked by potassium cyanate at 0.6 mM. Ethylnitroso-<sup>14</sup>C-urea was synthesised and this also yields <sup>14</sup>C-cyanate ions when incubated in growth medium. The presence of protein seems to catalyse the breakdown; for example at 37 °C the half life of ENU in physiological saline is 150 min, but this goes down to 15 min in growth medium containing serum.

At levels of nitrosamide below 0.3 mM, DNA synthesis and cell proliferation are affected but the cells remain viable. Figure 2 shows how potassium cyanate also mimics these effects.

The cyanate ions are effective by virtue of their ability to effect carbamylation reactions. When cell cultures are incubated with MNU or ENU radiolabelled with <sup>14</sup>C in the urea moiety, then the appearance of isotope in the TCA-insoluble material can easily be followed and is still increasing in a linear fashion after 6 h. In contrast, when cells are incubated with MNU of an identical specific activity, but labelled in the alkyl moiety, isotope incorporation into

Fig. 2 Cultures of foetal brain cells were set up as in Fig. 1. Before achieving confluency, 50-mm plates were refed with F10 medium containing 10% FCS and immediately treated with KCNO (●) or ENU (□) previously dissolved in warm ethanol. All plates received an identical volume of ethanol. *a*, 10 h after the addition of nitrosamide, triplicate plates at each nitrosamide concentration were pulsed for 1 h with 0.2 μCi ml<sup>-1</sup> <sup>3</sup>H-thymidine (>20 Ci mmol<sup>-1</sup>). After 3 washes with PBS, cells were scraped from the plate with a silicone policeman and treated with a 5% TCA solution. After a further wash with 5% TCA, the insoluble material was collected on a GFC filter. TCA-soluble counts did not vary between plates. The filters were counted in a liquid scintillation spectrometer (Tracerlab); the scintillant was 5 g PPO and 20 g of naphthalene in 1 l of dioxan. *b*, Duplicate plates of cells were set up as in *a* and after 4 d cells were removed by trypsinisation and the number of cells counted with a Coulter counter.





**Fig. 3** Fetuin (Sigma) at  $15 \text{ mg ml}^{-1}$  in PBS was treated with a solution of KCNO ( $100 \text{ mg ml}^{-1}$ ) to give a range of final concentrations of KCNO from 0 to  $10 \text{ mg ml}^{-1}$ . All solutions were made  $0.05 \text{ } \mu\text{Ci ml}^{-1}$  with respect to  $\text{K}^{14}\text{CNO}$  and solutions were incubated at  $37^\circ\text{C}$  for 5 h. Before and after the incubation, samples were taken and from the TCA soluble and TCA-insoluble counts the amount of carbamylation per mg protein was calculated. This is expressed in terms of carbamyl groups per fetuin molecule. Although gel electrophoresis reveals the presence of contaminating bands, a molecular weight of 46,500 has been assumed. The protein samples were exhaustively dialysed against PBS. Confluent plates of BHK cells were trypsinised and suspended in serum-less F10 and plated-out into 50 mm Petri dishes. The carbamylated fetuin preparations were then added to duplicate plates to give a final concentration of  $5 \text{ mg ml}^{-1}$ . After 16 h cells were viewed under an inverted microscope with phase contrast optics and cells were counted as "stretched" (that is, having adopted a bipolar morphology) or "unstretched" (that is, totally, spherical). Results are expressed as a percentage of stretched cells (●). After 3 d further plates were trypsinised and counted with a Coulter counter (□).

TCA-insoluble material is at least one order of magnitude lower. It was originally expected that this latter would reflect a low level of alkylation, but it was subsequently found that isotope incorporation was identical if the  $^{14}\text{C}$ -methylnitrosourea was first incubated in growth medium for 3 d. Thus it is more likely to reflect low level metabolic utilisation of methanol formed during decomposition.

While the amount of cell-associated protein in a 50-mm dish is usually  $50\text{--}75 \text{ } \mu\text{g}$  at confluency, the protein in the growth-supporting foetal calf serum (FCS) will be at least  $15,000 \text{ } \mu\text{g}$ . Carbamylation of serum proteins by nitrosamides or cyanate also takes place and a correspondingly higher number of counts become associated with serum proteins

than with cellular proteins. This carbamylation of serum protein was found to be very important since it could completely inactivate the serum in respect of its growth-promoting properties. Thus FCS treated at  $37^\circ\text{C}$  with  $10 \text{ mg ml}^{-1}$  KCNO for 4 h followed by exhaustive dialysis produces a chemically modified serum which fails to stimulate DNA synthesis and cell proliferation. FCS is an heterogeneous mixture of proteins and for this reason it is impossible to correlate loss of biological function with degree of carbamylation. We had previously shown<sup>15</sup>, however, that certain lines of established cells would grow well with a commercial preparation of the glycoprotein fetuin<sup>16</sup> as sole macromolecular source. BHK cells<sup>17</sup> grow well with  $5 \text{ mg ml}^{-1}$  fetuin. If the fetuin was carbamylated with ENU, MNU or cyanate, such growth-promoting activities were lost. Using radiolabelled cyanate, the loss of biological activity and the degree of carbamylation were compared (Fig. 3). Above three carbamyl groups per fetuin molecule, BHK cells show startling morphological changes.

BHK cells divide very rapidly and this allowed the demonstration of a very rapid effect of cyanate or nitrosamide. If the cells were in exponential growth then as little as 15 min exposure to one of the above agents could cause an inhibition of mitosis. Table 1 shows such an inhibition achieved with cyanate.

In the past, little attention has been paid to the fate of any part of the ENU or MNU molecule other than the alkyl moiety. But in the case of the more complex nitrosamides, some of which have proved useful chemotherapeutic agents, it has been shown that carbamylation of macromolecules by isocyanate species takes place<sup>18-20</sup>. Certainly in the case of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea, carbamylation of protein has been shown to be a far more extensive reaction than alkylation of macromolecules<sup>19,20</sup>.

This communication presents evidence that MNU and ENU, on decomposition in culture, give rise to a very bioactive molecule, namely the cyanate ion, which can affect several stages of the cell cycle. The effect of nitrosamides and cyanate on serum indicates that low levels of protein modification may alter biological function. This might also be true inside the cell and in view of its activity *in vitro*, it is now necessary to determine whether the cyanate ion is responsible for any of the effects of nitrosamides *in vivo*.

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**Table 1** Inhibition of mitosis of BHK cells by cyanate

Time of exposure to KCNO (min)	Increase in cell no. per plate $\times 10^{-5}$ after:			
	2 h	4 h	6 h	24 h
0	0.8	2.0	3.5	14.4
15	0	0.4	2.3	14.0
60	0	0	0.5	11.6

Replicate plates of BHK cells in exponential phase of growth were treated with  $0.5 \text{ ml}$  of a KCNO solution in PBS to give a final concentration of  $0.4 \text{ mg ml}^{-1}$ . At the end of the exposure-time cells were washed and refed with ordinary growth medium. Control cultures were treated with a similar volume of PBS and washed and refed after 1 h. At 1 h ( $t=0$ ) and intervals thereafter, duplicate plates were trypsinised and counted with a Coulter counter.

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## Isolation of immunogenic tumour cells by cell-affinity chromatography

TUMOUR-HOST relationships are determined by many variables<sup>1,2</sup>, and may be altered to favour the host by the use of chemically modified cells which act as specific immunostimulants<sup>3</sup>. Furthermore, modified cells have therapeutic value in mice with small tumours<sup>4,5</sup>. Several studies have shown that conventional methods followed by administration of immunostimulants are very effective in treating tumours and inducing tumour-specific immunity<sup>6-8</sup>. Combined chemotherapy and immunotherapy of L1210 leukaemia with neuraminidase-treated (VCN) tumour cells yielded a higher percentage of long term survivors than did chemotherapy alone<sup>6,7,9,10</sup>. The therapeutic effectiveness of VCN-treated cells depended on several variables<sup>10</sup>, including proper selection of immunogenic cells<sup>7,11</sup>.

We now report that highly immunogenic cells can be isolated from the parental tumour cell population by cell-affinity chromatography with concanavalin A (con A). Immunisation with these cells after chemotherapy resulted in complete remission of L1210 leukaemia.

L1210 cells (Arthur D. Little Co.) were maintained as an ascitic tumour in BDF<sub>1</sub> (C57BL/6×DBA/2) males and athymic (*nu/nu*) mice. The cell line has also been maintained as a suspension culture using RPMI 1640 medium, supplemented with 5% foetal calf serum and antibiotics.

Cell-affinity columns were prepared by covalent coupling of con A to nylon fibres with glutaraldehyde<sup>12</sup>. L-leucine was added to the reaction mixture to compete with, and thereby "space" the con A molecules along the fibre. This technique resulted in a fourfold decrease of bound protein compared with coupling in the absence of the amino acid. The preparation and properties of the con A columns will be outlined in detail elsewhere. Recognising that the binding of cells to the con A-nylon was probably mediated through multiple (and different) sugar linkages, it was decided to elute the cells from the column by the sequential addition of sugars that possessed a decreasing order of free-solution affinity for con A<sup>13</sup>. The elution profiles for L1210 cells grown in the BDF<sub>1</sub> host, in culture, or in the athymic host are summarised in Table 1. The two cell lines grown *in vivo* seemed to represent the extremes in chromatography, since few cells were released by sugars, and the cell lines were primarily composed of cells either binding or not binding to the con A-nylon.

The immunogenic properties of the isolated subpopula-

**Table 1** Subpopulations of L1210 tumour cells (%) as a function of growth conditions

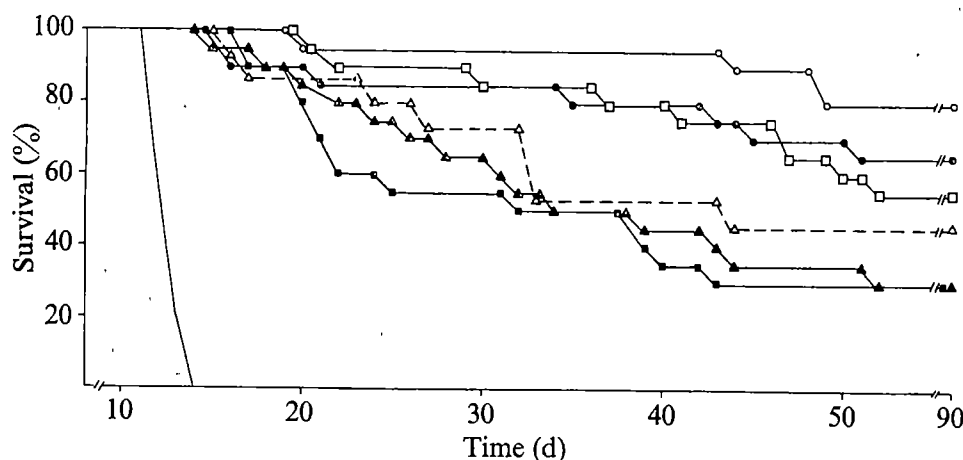
Cell fractions	<i>In vivo</i> (BDF <sub>1</sub> )	Growth conditions	
		<i>In vivo</i> (athymic)	<i>In vitro</i>
A	14.1	68.0	32.0
B	4.1	0.6	5.0
C	<0.01	0.2	0.7
D	<0.01	0.2	2.8
E	<0.01	0.03	3.9
F	81.8	30.6	56.6

Fractions A to F represent the percentage of total cells. A, eluted with phosphate-buffered saline (PBS), B, eluted with  $\alpha$ -methyl-D-mannoside; C, eluted with  $\alpha$ -methyl-D-glucoside; D, eluted with sucrose; E, eluted with  $\alpha$ -D-glucose; F, not eluted by the above sequence. Cells (ascitic tumours) grown *in vivo* were washed once in 1% ammonium oxalate, 25 °C, to lyse erythrocytes, followed by two washes in PBS. Cultured cells were washed twice in PBS. Preparation of *in vivo*-grown cells by Ficoll-Hypaque gradients<sup>14</sup> did not affect the elution profile. Affinity columns were prepared by packing 25-cm glass pipettes with brushed nylon (No. 3 denier, 1½ inch, type 200, DuPont) then incubated with equal volumes of 25% glutaraldehyde, 0.4  $\mu$ M L-leucine, and 1 mg con A (con A was Grade III, twice crystallised, all reagents Sigma), at pH 6.5 for 1 h at 37 °C. This procedure bound 1.9  $\mu$ g protein per g nylon. The column was then flushed with 50 volumes PBS, pH 7.2. Elution was at room temperature with a flow rate of 2 ml min<sup>-1</sup>.

tions were evaluated by their effectiveness in causing complete remission of L1210 tumour. The optimal protocol for this particular tumour-host model was developed by Kollmorgen *et al.*<sup>10</sup> and is based on initial reduction of tumour burden by chemotherapy. Immunotherapy consisted of a single injection of one of the different cell fractions. Survival after therapy correlated with the elution profile from the con A column (Fig. 1) with the best response observed in mice treated with fraction A. All surviving mice were immune to L1210 tumour when rechallenged with a lethal inoculum for virgin mice.

To further evaluate the immunogenic properties of these cells, the best and worst fractions (A and E, respectively) were treated with neuraminidase and used in an identical protocol. As Fig. 2 shows, the relative effectiveness of the two cell types was reversed after treatment with VCN, although long-term survival was similar. The early deaths observed in the group of mice immunised with VCN-treated fraction A cells suggests that chemotherapy with 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) had an immunological component which was abrogated, in this case, by immunotherapy.

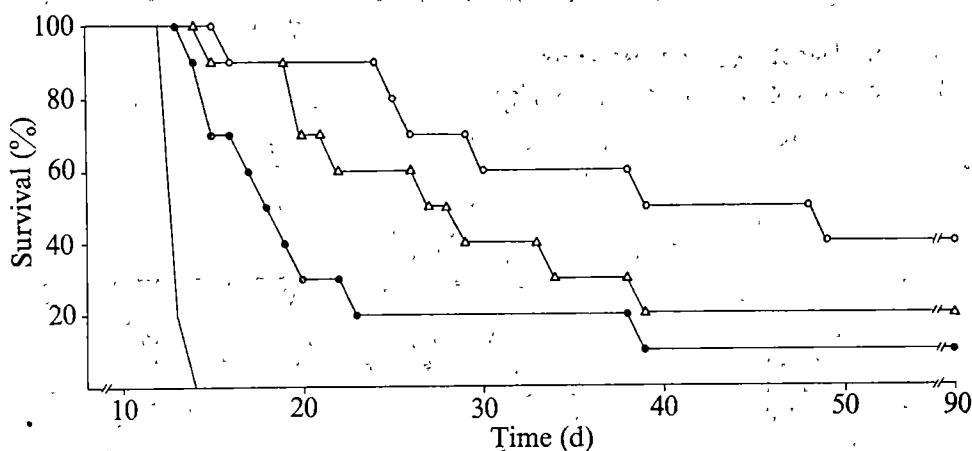
These results indicated that the immunogenicity of the different subpopulations was related to the expression of cell surface carbohydrates, as defined by con A-affinity



solid line indicates the survival of tumour controls (no treatment).  $\Delta$ , Chemotherapy with BCNU only;  $\circ$ , BCNU + fraction A;  $\square$ , BCNU + fraction B;  $\bullet$ , BCNU + fraction C;  $\blacktriangle$ , BCNU + fraction D;  $\blacksquare$ , BCNU + fraction E.

**Fig. 1** Percentage survival as a function of time after tumour injection. This protocol is based on optimisation of the L1210-BDF<sub>1</sub> tumour-host model<sup>10</sup> where mice received an intraperitoneal injection of  $10^4$  cells at time zero. The mice were randomised on day 6.5 (estimated tumour burden  $10^8$  cells) and all but tumour controls received a single intraperitoneal injection of BCNU (12 mg kg<sup>-1</sup>) to reduce tumour burden. The mice were again randomised on day 8, and immunotherapy consisted of a single intraperitoneal injection of  $10^4$  cells (inactivation of the cells by one freeze-thaw generally resulted in less than 10% viability). Each group contained 20 mice. The

**Fig. 2** Percentage survival as a function of time after tumour injection. The experimental protocol is identical to that described in Fig. 1, except that fraction A and fraction E cells were treated with VCN (Grand Island Biological). The cells were concentrated by centrifugation and incubated with equal parts acetate buffer, pH 5.6 and VCN (1 U per  $10^6$  cells) for 1 h at  $37^\circ\text{C}$ . The cells were washed twice with 5 mM EDTA-saline and stored in PBS at  $-90^\circ\text{C}$  until use. Each group contained 10 mice. The solid line indicates the survival of tumour controls (no treatment).  $\Delta$ , Chemotherapy with BCNU only;  $\circ$ , BCNU + VCN-treated fraction E;  $\bullet$ , BCNU + VCN-treated fraction A.



chromatography. Further studies were designed to examine the nature of L1210 cell antigenicity. Mice were immunised with VCN-treated, cultured L1210 cells in the combination therapy protocol previously described. The survivors were boosted with  $10^6$  viable L1210 cells (to which they were immune) and bled 7 d later. The serum was then tested for complement-dependent cytotoxicity against cultured L1210 cells, in the presence or absence of various sugars. Cytotoxicity was measured by the  $^{51}\text{Cr}$ -release assay<sup>14</sup>, and the results are expressed as mmol of sugar required to cause 75% inhibition of cytotoxicity (Table 2). Of the sugars used to elute cells from the con A column, the most effective inhibitor of cytotoxicity was  $\alpha$ -methyl-D-mannoside, the sugar which released fraction B. The poorest inhibitor was  $\alpha$ -D-glucose, which released fraction E. Galactosyl-like residues have been considered as part of the binding site for "naturally-occurring" cytotoxic antibodies to VCN-treated cells<sup>15,16</sup>. Galactose and galactosamine were non-inhibitors in the study reported here, although the non-reducing sugar  $\alpha$ -methyl-D-galactoside was an effective inhibitor of cytotoxicity. Maximal inhibition of cytotoxicity was observed using fucose. Preliminary studies have shown that the percentage of cells released from fucose-specific lectin columns was three to five times higher for fraction A than for the whole tumour cell population (J.J.K., unpublished). Experiments are in progress to evaluate the immunogenic properties of tumour cells isolated by fucose-specific affinity columns.

Edelman *et al.* have shown that antigen-specific lymphoid cells can be isolated by their binding to antigen-derived

fibres<sup>17</sup>. The use of their techniques<sup>17-19</sup>, in combination with the chromatography of tumour cells described here, offers an excellent system for study of interactions between lymphoid and tumour cells.

Tumour cell populations are heterogeneous with respect to various biological<sup>20-22</sup> and antigenic properties<sup>23,24</sup>. This heterogeneity may provide clones with different membrane characteristics and nutrient requirements, and thereby influence the rate of tumour growth and extent of metastatic involvement. Metastasis may be mediated by cell adhesion<sup>25</sup>, which in turn, may be a function of cell surface proteases, glycosidases and charge density<sup>26-28</sup>. Cellular interaction of blood-borne tumour cells with lymphocytes<sup>29</sup> and platelets<sup>30</sup> can also affect the distribution of circulating tumour cells. Different growth conditions may also result in the alteration of membrane properties<sup>31</sup>, immunogenicity<sup>7,32</sup> and metastatic properties of tumour<sup>33</sup>.

These observations, together with the results reported here have important implications for the immunotherapy of cancer. We have reported<sup>11</sup> that immunotherapy was successful only when cells used for immunisation elicited immunological products that were cytotoxic for residual tumour. Animals with drug-resistant tumour required immunisation with drug-resistant cells for optimal therapeutic benefit (J.J.K., unpublished). Drug-resistant sublines expressed marked alterations in antigenicity<sup>34</sup> and serum from mice cured by immunisation with VCN-treated, drug-resistant cells had minimal cytotoxicity directed against the parental cell line<sup>11</sup>.

In the treatment of human cancer, the probable source of autologous tumour cells for subsequent immunotherapy will be preserved specimens of the primary tumour. These cells may bear little antigenic resemblance to tumour cells which emerge during treatment.

The results reported here suggest that a new approach to active, specific immunotherapy may be developed—to "antigenically match" residual tumour with isolated sub-populations from the primary (parental) neoplasm. This will require that predictive relationships be established between the antigenic properties of metastatic and drug-resistant tumour cells, and the antigenic properties of isolated sub-populations from parental tumour. In L1210 leukaemia, the immunogenic fraction A expanded when the cell line was cultured, and was the dominant cell type when tumour was grown in athymic mice, suggesting an excellent source for immunogenic cells.

Studies are in progress to extend this concept of immunotherapy to other tumour-host models, and to the therapy of antigenically distinct clones of tumour cells that may emerge after treatment with chemotherapeutic agents.

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**Table 2** Carbohydrate-inhibition of complement-dependent serum cytotoxicity to cultured L1210 cells

Inhibiting carbohydrates	mM required for 75% inhibition
$\alpha$ -D-Glucose	50.00
Sucrose	1.00
$\beta$ -D-Glucose	0.30
$\alpha$ -Methyl-D-galactoside	0.01
$\alpha$ -Methyl-D-mannoside	0.007
$\alpha$ -D-Fucose	0.001
$\alpha$ -D-Fucose	0.001
Non-inhibiting carbohydrates	
D-(+)-Galactose	
D-(+)-Galactosamine	
N-Acetyl-D-glucosamine	
$\alpha$ -Methyl-D-glucoside	

Mice were treated in an identical experimental protocol as described in Fig. 1, except immunotherapy consisted of VCN-treated, cultured L1210 cells. The serum of surviving mice were pooled and cytotoxicity was determined by  $^{51}\text{Cr}$  release<sup>14</sup>. Serum was preincubated with sugars for 30 min before addition of target cells. Serum cytotoxicity without inhibitor was 40% of total releasable  $^{51}\text{Cr}$ , and all inhibitors were tested from 300 mM to 10 pmol.



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## Differential response of cells grown in light and dark to near-ultraviolet light

We have previously shown that near-ultraviolet and visible light (290-650 nm) kill mitotic and early S phase amoebae, whereas G<sub>2</sub> phase amoebae are quite resistant<sup>1</sup>. Exposure of amoebae to near-ultraviolet light alone (290-400 nm) can have similar results (our unpublished data). We report here variations in sensitivity to near-ultraviolet irradiation of amoebae grown in 12 h light/12 h dark (hereafter referred to as light-grown cells) and those grown in complete darkness. We also show that in the dark-grown cells, as well as the S phase of the light-grown cells, the repair mechanism against the induced lethal damage might be lacking or non-functional.

*Amoeba proteus* were cultured at 21 ± 1 °C, as before<sup>2</sup>. Both light-grown and dark-grown cells have a generation time of 36 ± 4 h, and an S period of 6-7 h with no G<sub>1</sub> (ref. 3). The dark-grown cells (originally from a light-grown culture) had been kept in an incubator at 20 °C for about 22 months. They were exposed briefly to light when the culture dishes were changed every third day. The intensity of light incident on the light-grown cell cultures was ~ 5 W m<sup>-2</sup> from fluorescent tubes. The amoebae grew equally well when moved from dark to light and vice versa.

Light-grown and dark-grown amoebae were exposed at different phases of the cell cycle to near-ultraviolet irradiation at an intensity of 14 W m<sup>-2</sup> for various periods. The survival curves (Fig. 1) show that both the S and the G<sub>2</sub> stages of the dark-grown cells (DS and DG<sub>2</sub>) as well as the S phase of the light-grown cells (LS) were killed within 5-7 d at an energy

fluence of ~ 1.5 × 10<sup>5</sup> J m<sup>-2</sup> of near-ultraviolet light. This dose also killed all the mitotic cells from light-grown and dark-grown cultures (not shown). In contrast, the G<sub>2</sub> phase amoebae from light-grown cultures (LG<sub>2</sub>) were highly resistant to this dose of near-ultraviolet light, although a division delay (between 48-96 h) was noted. In controls, the survival rate was > 99%.

To assess the sensitivity of the nucleus and cytoplasm of the amoebae to near-ultraviolet light, nuclear transplantation experiments (according to the method of Jeon and Lorch<sup>4</sup>) were undertaken. Amoebae exposed to the lethal dose (1.5 × 10<sup>5</sup> J m<sup>-2</sup>) of near-ultraviolet light were selected for nuclear transplantation studies. All nuclear transfers were carried out almost immediately after exposure and completed within ~ 30 min. In most cases, both the nucleus and the cytoplasm of dark-grown and near-ultraviolet-exposed cells failed to survive when recombined with the cytoplasm and nucleus respectively from control LG<sub>2</sub> or DG<sub>2</sub> phase cells. When the nucleus from a light-grown and near-ultraviolet-exposed S phase cell was grafted into the control LG<sub>2</sub> phase cytoplasm a partial recovery (~ 30%) of the treated nucleus was observed. Most of the treated cytoplasm from the light-grown cells could, however, be rescued when the nucleus from control LG<sub>2</sub> cell was implanted into them (see Table 1).

These experiments show that: first, the control LG<sub>2</sub> and DG<sub>2</sub>

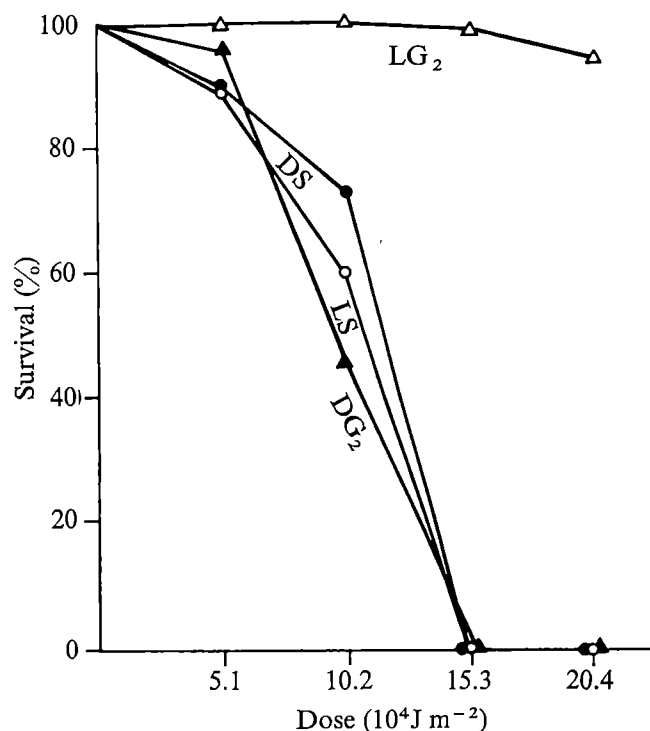


Fig. 1 Survival curve of near-ultraviolet exposed light-grown and dark-grown amoebae at different phases of the cell cycle. LS, LG<sub>2</sub>, S phase and G<sub>2</sub> phase of light-grown amoebae; DS, DG<sub>2</sub>, S phase and G<sub>2</sub> phase of dark-grown amoebae. The amoebae at known cell cycle stages, (S phase, 0-1-h-old cells; G<sub>2</sub> phase 18-24-h-old cells), obtained by mechanical selection of the division spheres, were placed in a specially constructed watch glass, partitioned into four chambers. A Reichart UG-1 filter with < 1% transmission below 280 nm and above 410 nm, and having a maximum transmission of 73% at 360 nm, was placed on the top of the watch glass. The light source used was a 400-W Philips white fluorescent lamp<sup>1</sup> and the amoebae of different age groups were exposed simultaneously to near-ultraviolet light transmitted through the filter at an intensity of 14 W m<sup>-2</sup> as measured by a Schwarz linear vacuum thermopile. The temperature of the culture medium during the exposure was maintained at ~ 20 °C by means of a water-circulating thermostat (Lauda). The cells were washed after exposure with medium and kept in syracuse watch glasses together with food organisms. No difference in response was noted between cells kept in total darkness and those kept in a 12 h light/12 h dark cycle after the exposure. Between 50 and 150 amoebae were exposed for each experimental set.

**Table 1** Results of nuclear transplantation experiments between near-ultraviolet treated and control amoebae from light-grown and dark-grown cultures at different cell cycle stages

Type of transfer	No. of transplants	No. of cell deaths	Survival† (%)
$A(D)S_n^* \rightarrow (D)S_n \leftarrow \left\{ (L/D)G_c \right.$	69 38	67 4	3 90
$(D)S_c^* \leftarrow (D)S_c \leftarrow \left\{ (L/D)G_n \right.$	65 36	59 3	9 91
$B(L)S_n^* \rightarrow (L)S_n \rightarrow \left\{ (L)G_c \right.$	15 17	10 1	33 94
$(L)S_c^* \leftarrow (L)S_c \leftarrow \left\{ (L)G_n \right.$	15 14	1 2	93 86

\*Near-ultraviolet treated cells.  $n$ ,  $c$ , indicate the nucleus and the enucleated cytoplasm respectively.

S, S phase (2–3 h after mitosis); G, G<sub>2</sub> phase (18–20 h after mitosis). (D), Dark-grown cells; (L), light-grown cells; (L/D), light or dark-grown cells.

†Production of at least sixteen amoebae from a single transplant has been taken as an index of survival.

nuclei cannot repair near-ultraviolet-induced damage of the DS cytoplasm. Second, the control LG<sub>2</sub> nucleus can repair the damage of the treated LS cytoplasm. Third, the treated LS nucleus can partially recover when transferred into the control LG<sub>2</sub> cytoplasm, but the treated DS nucleus cannot recover when grafted into either the LG<sub>2</sub> or DG<sub>2</sub> control cytoplasm.

The cytoplasm and the nucleus of dark-grown cells both seem to be highly sensitive, whereas the nucleus of light-grown S phase cells is partially, and the cytoplasm fully, resistant to near-ultraviolet light. Furthermore, our data strongly suggest the presence of a recovery factor(s), for example, a repair enzyme against the near-ultraviolet-induced damage in light-grown G<sub>2</sub> phase amoebae, which might be lacking or non-functional in the other cases mentioned here (see also ref. 1). It is interesting that an LS nucleus exposed to near-ultraviolet light can partially recover in the control LG<sub>2</sub> cytoplasm, while the treated DS nucleus shows insignificant recovery in either LG<sub>2</sub> or DG<sub>2</sub> control cytoplasm. Thus, it seems that repair against damage induced by near-ultraviolet light involves, as well as a recovery factor(s) in the LG<sub>2</sub> cytoplasm, a capacity of the nucleus of the exposed cell to augment recovery—which is probably absent in the dark-grown cells.

Near-ultraviolet light induces thymine dimer formation, DNA single-strand breaks, and also inactivates photoreactivating enzyme. Because of the poor absorption of DNA and proteins in this region of the spectrum, it has been suggested that the mechanism of induction of such damage is similar to that of photodynamic effect, being mediated through an endogenous sensitiser, for example, riboflavin or ubiquinone (for review see ref. 5).

We suggest that a certain region of the visible light spectrum can repair, directly or indirectly, the damage done by 360-nm light or that it is antagonistic to the deleterious action of this light before the fixation of the damage in the cell. The reasons are as follows: first, cells exposed to near-ultraviolet light are killed much earlier (5–7 d) than those exposed to visible light (3–5 weeks)<sup>1</sup>. Second, almost an equivalent dose ( $1.5 \times 10^5 \text{ J m}^{-2}$ ) of 350–370-nm light was received by both the cells exposed to visible light<sup>1</sup> and to near-ultraviolet light, resulting in 100% lethality. At this dose, light-grown S phase nuclei exposed to visible light show almost complete recovery (88%) when transplanted into the control G<sub>2</sub> phase cytoplasm, compared with partial recovery (33%) when exposed to near-ultraviolet light.

There are some reports that growth in darkness has changed or modified cellular functions. *Nicotiana tabacum* plants, kept

in the dark, lose their ability to photoreactivate ultraviolet-treated tobacco mosaic virus RNA within a week. Such plants recover their photoreactivation ability after they are returned to the light<sup>6</sup>. Mutants have been obtained from *Rhodospirillum rubrum* cells, grown for > 100 generations in the dark, which are light-sensitive and produce only a trace amount of bacteriochlorophyll *a* (ref. 7).

Finally, it would be interesting to know the timings of the 'switch over' from resistance to sensitivity to near-ultraviolet light and vice versa in the amoebae. Our preliminary experiments suggest that dark-grown G<sub>2</sub> phase cells acquire resistance to near-ultraviolet light in 10 d, or less, after they are returned to the light. The induction of this resistance thus seems to be photoregulated.

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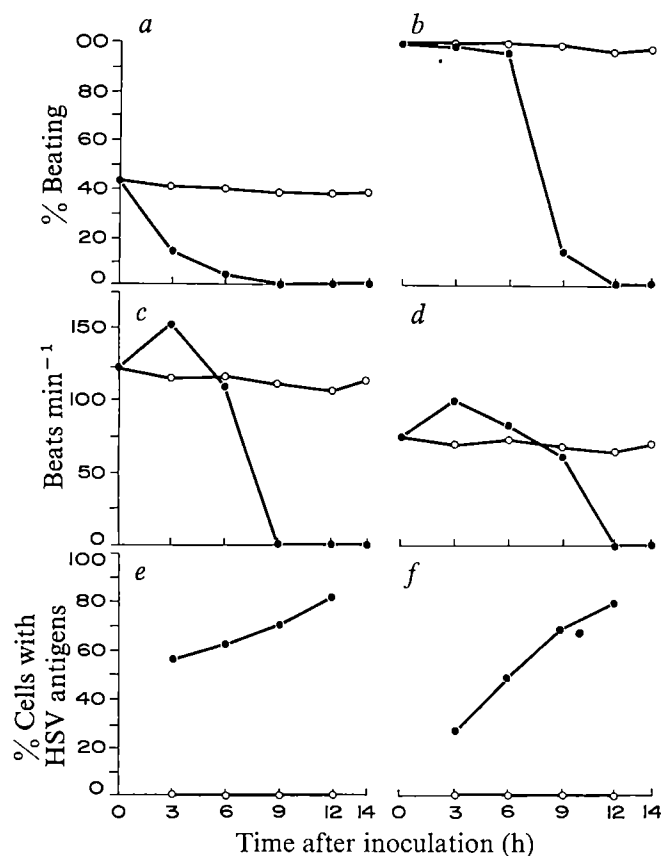
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## Herpes simplex virus infection stops spontaneous beating of chick heart cells

PROFOUND modifications in the macromolecular structure of cell membranes and membrane-related functions have been demonstrated on infection with various viruses<sup>1,2</sup>. For example, shortly after infection with certain herpesviruses, synthesis and insertion of host proteins into plasma membranes cease and new virus-specified proteins begin to appear<sup>3,4</sup>. These viral proteins differ from the cell membrane proteins in size, electrophoretic mobility, extent of glycosylation and sulphate incorporation<sup>3–5</sup>. The changes in membrane structure are reflected in altered morphology, antigenic characteristics and contact interactions of herpesvirus-infected cells<sup>4,6</sup>. They also appear to affect the ion-dependent electrical properties of the cells as shown by alterations in the transmembrane potential of cells infected by herpesviruses *in vitro* and *in vivo*<sup>7,8</sup>. To elucidate further alterations in membrane-related cell functions, as well as virus-cell interactions associated with herpesvirus infection, we are studying the effects of herpes simplex viruses (HSV) type 1 and 2 on chick embryo heart cells, which provide a useful system, particularly when aggregated into spontaneously beating spheroidal clusters<sup>10–13</sup>. We found that these viruses stopped the spontaneous beating of the cells before cell 'death'.

Cell monolayers were prepared from 7-d-old chick embryo hearts by a multicycle trypsinisation procedure described before<sup>10</sup>. Dishes (35 mm diameter) containing 2 ml of medium 818A were seeded with  $2 \times 10^5$  cells per dish and incubated at 37 °C in a water-saturated atmosphere of 5% CO<sub>2</sub>, 10% O<sub>2</sub> and 85% N<sub>2</sub>. Cell aggregates were prepared from the dissociated heart cells by incubating Erlenmeyer flasks (50 ml) containing 10 ml of medium and  $1 \times 10^6$  cells per flask (gassed with 5% CO<sub>2</sub>) at 37 °C on a shaker gyrating at 75 r.p.m. (ref. 11). After 18–20 h of incubation, cultures were washed and examined on the constant-temperature (37 °C) stage of an inverted microscope, in 5% CO<sub>2</sub> atmosphere.

Heart cell monolayers and aggregates were inoculated for most experiments with HSV-1 (VR<sub>3</sub> strain) at multiplicities of infection (MOI) between 0.25 and 200 plaque forming units (PFU) per cell. After 90 min of viral adsorption at 37 °C, the preparations were washed with fresh



**Fig. 1** Embryonic chick heart cell monolayers (*a*, *c* and *e*) and aggregates (*b*, *d* and *f*) inoculated with herpes simplex virus (●) or mock inoculated with inactivated virus (○). *a* and *b*, Percentage of heart cells in monolayers or aggregates beating. *c* and *d*, Beating rate (beats per min). *e* and *f*, Percentage of cells exhibiting HSV antigens by immunofluorescence in monolayer or aggregated heart cells.

medium and reincubated as before. To confirm that cultures were infected, supernatant medium or homogenates of the cells were subinoculated into cultures of primary rabbit kidney cells. In addition, HSV antigens were detected in the virus-inoculated heart cells by a direct immunofluorescent technique<sup>14</sup>. Heart cultures mock inoculated with virus inactivated at 56 °C for 3 h served as controls.

HSV infection of heart cells in monolayer culture caused the cells to round up within 6–12 h and stop beating. In a typical test, the number of beating cells in monolayers inoculated with virus (MOI=100) fell from 43% at the time of inoculation to 4% 6 h later (Fig. 1*a*). Only a few cells beat arrhythmically and with variable rates 9 h after inoculation. At 12 h, none were beating; contractions were not restored by a change of medium. No significant alterations were observed in the beating activity of cells mock inoculated with heat-inactivated virus.

When the virus inoculum was added to cell suspensions on a gyratory shaker at the beginning of aggregation, cells either failed to aggregate or formed irregular clumps of loosely attached cells. For this reason, preformed spheroidal aggregates were inoculated with HSV after 24 h of gyration. Rounding of cells occurred at the periphery of the aggregates. At the time of inoculation, 98–100% of the aggregates beat, but only 14% continued beating 9 h later; none of the aggregates beat 12 h after inoculation (Fig. 1*b*). Preliminary recordings of aggregates impaled with intracellular electrodes at intervals after HSV infection indicated that action potentials ceased and membrane resting potential declined slightly when the beat stopped.

The mean beating rate of cells and aggregates increased

by 25–30% over the control rate 3 h after inoculation, and then declined gradually before beating stopped (Fig. 1*c* and *d*). Mock inoculated control aggregates continued to pulsate without significant change in rate. The transient increase in beating rate after virus inoculation resembles the initial increase in beating rate of aggregates partially depolarised by ultraviolet light<sup>15</sup>, and may be attributable to depolarisation.

Inoculation with the VR<sub>3</sub> strain of HSV-1 in a range of MOI between 0.25 and 200 PFU per cell demonstrated that an MOI greater than 2.5 was required to stop the beating of either single cells or aggregates within 24 h. Infection with two other HSV-1 strains (F and HEA) at MOI of 100 produced effects on the heart cells similar to those observed with the VR<sub>3</sub> strain, while two HSV-2 strains (MS and G) stopped the beat at the same time with an MOI of 20.

Three hours after inoculation with the HSV-1 (VR<sub>3</sub>) at an MOI of 100, viral antigens were detected by immunofluorescence in about half the cells in monolayer culture, when the percentage of beating cells had dropped from 45 to 18% (Fig. 1*a* and *e*). Thereafter, as the number of beating cells continued to fall, there was a concomitant increase in the percentage of HSV antigen-positive cells. In contrast, 95% of the aggregates continued to beat 6 h after inoculation, when about half of their constituent cells exhibited viral antigens (Fig. 1*b* and *f*). The number of beating aggregates declined abruptly to 15% 3 h later, when antigen-positive cells had increased to 70%. The difference in response between monolayer cells and aggregates may be attributed to the depolarisation in the tightly coupled aggregates<sup>11,12</sup>, as well as to a difference in the rate of viral multiplication in aggregated cells as compared to monolayer cells due to the differences in topography.

It is noteworthy that 12 h after infection, at the time all cells and aggregates had stopped beating, 92% of the cells in monolayers were found to exclude Trypan blue dye. Similarly, when the 12-h-infected aggregates were dissociated with trypsin, 95% of their component cells excluded the dye. This indicates that viral infection at this time had not caused the cell membranes to become permeable to molecules the size of Trypan blue, and suggests further that the loss of beat and electrical activity did not result merely from non-selective leakiness of the cell membranes. Although virus-infected cells eventually die, the brief interval (3–6 h) between viral inoculation and the effects on beating rate reported here argues for more subtle alterations in the infected cell membranes as the underlying cause of these physiological changes. More work is needed to discover how such changes are correlated with those that have been observed with biochemical and immunological methods at the same early time interval<sup>4,8</sup>.

As far as we know, no earlier investigations of the pathological effects of viruses on embryonic heart cells have been reported, except that such cells infected with mumps virus in conditions somewhat different from those we used, continued beating<sup>16</sup>.

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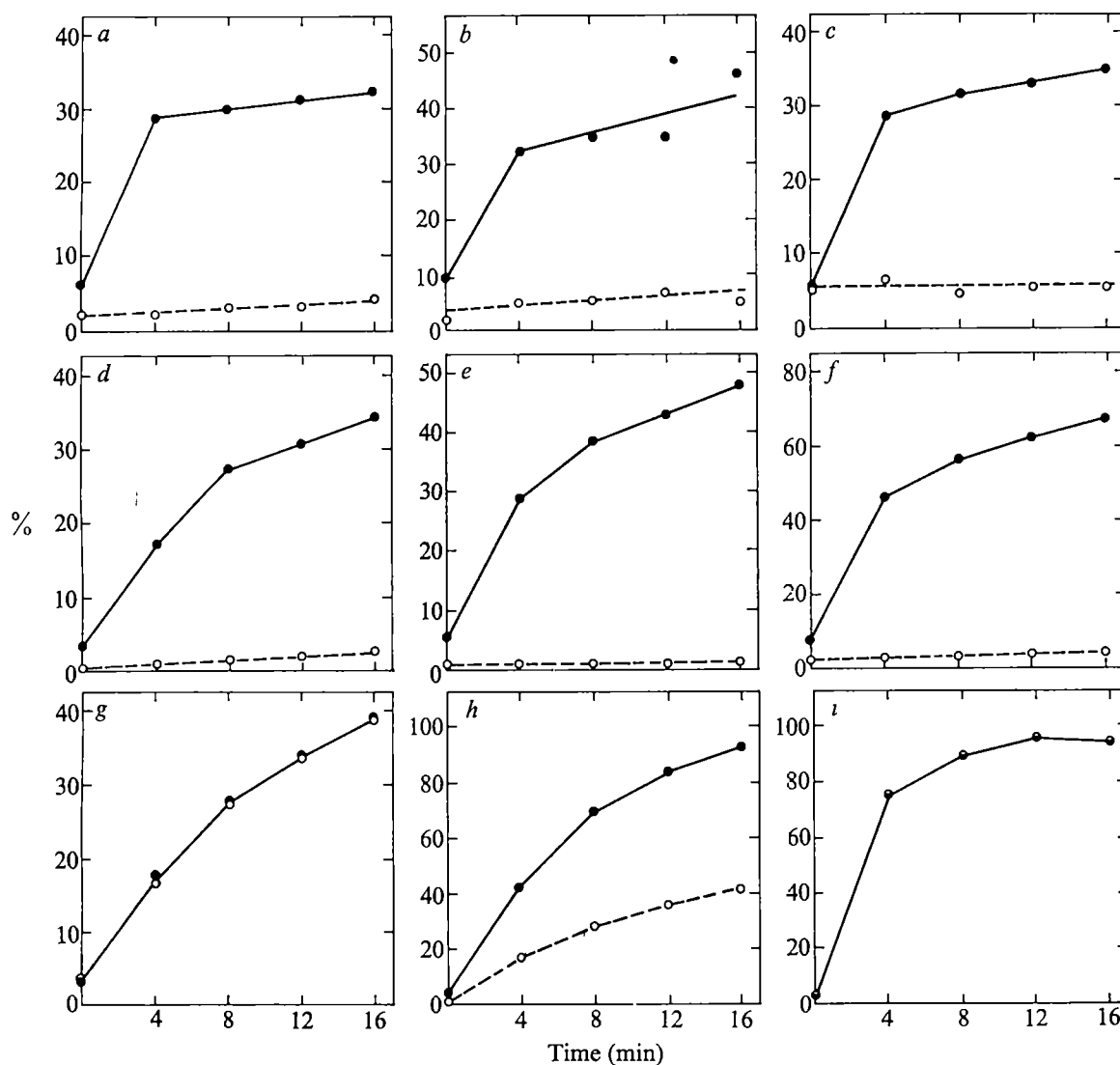
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## Unrelated animal viruses share receptors

THE specific receptors for several non-enveloped viruses are present on cells in only limited numbers ( $1 \times 10^4$ – $10 \times 10^4$ ), and can therefore be saturated with excess virus<sup>1-5</sup>. An

excess of inactivated poliovirus type 1 inhibits the attachment of infectious poliovirus of all 3 serotypes, but not the attachment of other viruses, including B Coxsackie viruses<sup>6</sup>. Most of the B Coxsackie viruses probably share the same receptor<sup>7</sup>, but Coxsackie viruses B1 and B3 do not compete for the poliovirus receptor<sup>1</sup>. Adenovirus 2 and 5 also appear to share the same receptor, since their attachment to host cells is blocked by an excess of the adenovirus type 2 fibre protein, which seems to be the receptor-recognising portion of the virus particle<sup>2</sup>. A number of other adenoviruses may also belong to the same receptor family since soluble antigens block their attachment to erythrocytes<sup>8</sup>. We here report results which permit the construction of a 'receptor map' for a number of non-enveloped viruses.

These experiments measured attachment of highly purified <sup>14</sup>C-, <sup>35</sup>S-, or <sup>32</sup>P-labelled virus particles to HeLa cells. The HeLa cells had either not been exposed to another virus, or exposed to excess purified virus or viral antigen to saturate most of the available receptor sites of one type. Pretreatment with excess virus was usually for 15 min at 34.5 °C



**Fig. 1** Attachment of labelled virus particles to untreated cells (●) or to cells treated with excess unlabelled CB3, Ad2 or Ad2-fibre protein (○). Attachment of <sup>14</sup>C-CB3 was inhibited by excess homologous virus (a), and by excess Ad2 (b) or by excess Ad2 fibre protein (c). Attachment of <sup>35</sup>S-Ad2 was inhibited by excess CB3 (d), by excess homologous virus (e) or excess fibre (f). CB3 did not inhibit attachment of CA21 (g). Excess Ad2 particles partly inhibited attachment of HRV-2 (h) but the fibre protein did not (i). Non-radioactive virus ( $1 \times 10^5$ – $3 \times 10^5$  particles per cell) or Ad2 fibre protein ( $8 \mu\text{g ml}^{-1}$ ) was incubated for 15 min at 34.5 °C with HeLa cells ( $1 \times 10^7 \text{ ml}^{-1}$ ), or cells were 'mock' incubated without added virus or fibre. The suspensions were cooled to 0 °C and a labelled virus was added ( $\sim 1\%$  by volume,  $1 \times 10^4$  particles per cell). Aliquots of cells were incubated at 34.5 °C, washed twice with cold medium and counted. Attachment of labelled virus is expressed in % of total added. Preparations of virus were usually dialysed against the MEM medium used for the cell suspension (adenovirus was in a buffer containing 10% glycerol, 0.001 M  $\text{MgCl}_2$ , and 0.02 M Tris-HCl, pH 7.8; this was also used with 'mock' treated cells. Adenovirus fibre protein ( $2 \text{ mg ml}^{-1}$ ) was purified by chromatography on DEAE cellulose, hydroxylapatite and Sepharose 6B<sup>11</sup>. The method of preparing, handling and counting the radioactivity of the cells has already been described<sup>3</sup>. 'Rhino-HeLa cells' were used in panels c, f, and i.



(Fig. 1), but 60 min at 22 °C or no pre-incubation but using a larger excess of unlabelled particles, gave compatible results (not shown). Excess unlabelled human rhinovirus type 2 (HRV-2) and type 14 (HRV-14), Coxsackie virus type A21 (CA21) and type B3 (CB3), poliovirus type 2 (P2), and adenovirus type 2 (Ad2) were employed for saturation of cellular receptors. These and other viruses were prepared and purified by methods already reported<sup>2,3,9,10</sup>. The concentration of picornaviruses<sup>10</sup> or adenoviruses<sup>2</sup> was estimated in particles per ml by ultraviolet adsorption. Pools of purified virus contained  $1 \times 10^{13}$ – $3 \times 10^{13}$  particles per ml, and when labelled,  $0.6 \times 10^8$ – $6 \times 10^8$  c.p.m. per ml.

Two lines of suspension-grown HeLa cells were used. The human rhinoviruses and Coxsackie virus A21 appeared to attach significantly more rapidly to one of these, 'rhino-HeLa-cells' than to the other, but the two lines otherwise showed no qualitative differences in competition experiments.

Efficient attachment of radioactive virus to cells required warming of the mixture. The rate of attachment varied from one type of virus to another and from experiment to experiment depending upon the cell line employed. Homologous competition for receptors was tested and detected in every experiment using excess virus or antigen.

Figure 1 illustrates representative results of experiments employing excess CB3, Ad2, or Ad2-fibre. As expected, attachment of <sup>14</sup>C-labelled CB3 was inhibited by excess homologous virus (Fig. 1a), but unexpectedly, attachment of CB3 was also inhibited by excess Ad2 (Fig. 1b) or Ad2-fibre (Fig. 1c). In a reciprocal way, attachment of <sup>35</sup>S-Ad2 was blocked by excess CB3 (Fig. 1d) as well as by excess Ad2 particles or fibre protein (Fig. 1e and f). Thus, CB3 and Ad2 clearly share HeLa cell receptors.

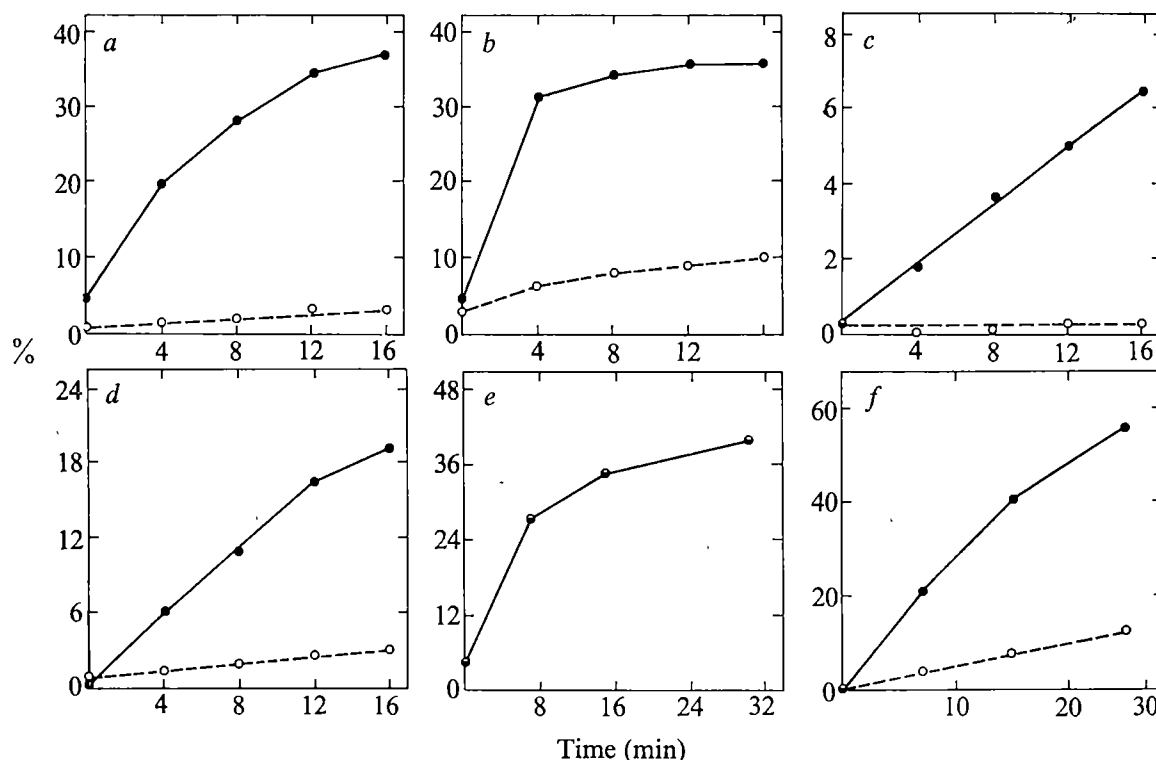
Excess unlabelled CB3 did not inhibit attachment of labelled HRV-2, HRV-14, P2 (not shown) or labelled CA21 (Fig. 1g). A different picture emerged with unlabelled Ad2 which produced a clear twofold reduction in the rate of attachment of labelled HRV-2 (Fig. 1h). This was not

reciprocal, however, and excess HRV-2 had no effect on attachment of labelled Ad2 (not shown). Treatment of cells with the relatively small (molecular weight 200,000 (ref. 11)) Ad2 fibre protein had no effect on HRV-2 attachment (Fig. 1i), indicating that the large bulky Ad2 particles on one site may interfere with attachment of HRV-2 to a separate site by steric hindrances. Alternatively, adenovirus may induce cross linking of components of the cell surface, which may lead to alteration or internalisation of the receptors for HRV-2. HRV-2 and Ad2 may thus recognise either separate determinants on the same macromolecule or determinants on adjacent macromolecules. It would be interesting to determine if isolated purified receptors for Ad2 will interact with HRV-2 particles as well. Cells treated with excess Ad2 also showed a barely detectable (~10%) reduction in attachment of HRV-14, CA21, and P2 (not shown).

Figure 2 shows some of the effects of treating cells with excess CA21 or excess HRV-14 on the attachment of other viruses. Excess CA21 is able to inhibit attachment of both labelled homologous virus (Fig. 2a) and also labelled HRV-14 (Fig. 2c). Competition for receptors by excess HRV-14 was more difficult to demonstrate, probably because HRV-14 attaches slowly to HeLa cells<sup>3</sup>. Thus cells treated with excess HRV-14 attached significant amounts of CA21 or HRV-3 (Fig. 2b and f), but there was clear evidence for interference. Similar data have been found for the attachment of HRV-41 (ref. 3) and some other labelled rhinoviruses (HRV-5, 15, 39, 51) to cells treated with excess HRV-14, and they probably all share the same receptor with the poorly attaching HRV-14. It is interesting to note that although the B Coxsackie viruses also probably share one receptor, the rates of attachment differ widely and show entirely different pH optima<sup>12</sup>. Neither CA21 nor HRV-14 were able to inhibit attachment of HRV-2, P2, CB3, or Ad2 (not shown, see also ref. 3 and Fig. 2e).

Our results are summarised in Table 1. There are four receptor families. The first family contains HRV-2,

**Fig. 2** Attachment of labelled virus particles to untreated cells (●) or to cells treated with excess unlabelled CA21 or HRV-14 (○). Attachment of <sup>14</sup>C-CA21 was inhibited by excess CA21 (a) or excess HRV-14 (b). Attachment of <sup>14</sup>C-HRV-14 was inhibited by excess CA21 (c) or HRV-14 (d). <sup>32</sup>P-P2 was not inhibited by excess CA21 (e); HRV-3 was inhibited by excess HRV-14 (f). The method used is described in the legend to Fig. 1. 'Rhino-HeLa cells' were used in panels b, d, e, and f.



**Table 1** Virus receptor families detected by competition for attachment to HeLa cells\*

In presence of excess	HRV-2	HRV-14	CA21	P2	CB3	Ad2
HRV-2†	+	—	—	—	—	—
HRV-14‡	—	+	+	—	—	—
CA21	—	+	+	—	—	—
P2	—	—	—	+	—	—
CB3	—	—	—	—	+	+
Ad2	±§	(-)	(-)	(-)	+\$	+

\*Strains of virus used: HRV-2(HGP), HRV-14(1059), CA21(48654), P2 (P217-Ch2ab), CB3 (Nancy), Ad2 (Adenoid 6), HRV-1A (2060), HRV-1B (B-632), HRV-3 (FEB), HRV-5 (Norman), HRV-15 (1734), HRV-39 (209), HRV-41 (56110), HRV-51 (F01-4081).

†In addition to competition (+) with attachment of homologous virus, excess HRV-2 also inhibited attachment of HRV-1A and HRV-1B (ref. 3, and Lonberg-Holm, unpublished data). Excess HRV-14 did not inhibit attachment of HRV-1A or HRV-1B.

‡In addition to the homologous and reciprocal inhibitions (+) indicated, excess HRV-14 also inhibited attachment of HRV-3, -5, -15, -39, -41, and -51. Excess HRV-2 did not inhibit these viruses (ref. 3, and Lonberg-Holm, unpublished data).

§Adenovirus fibre protein and intact Ad2 blocked attachment of CB3. Intact Ad2, but not excess fibre, partially inhibited attachment of HRV-2.

||Attachment of HRV-14, CA21 and P2 was inhibited by ~10% following treatment of cells with excess Ad2.

HRV-1A and HRV-1B. The second family contains HRV-14 and CA21. Other experiments, with excess HRV-14, indicate that HRV-3, -5, -15, -39, -41 and -51 belong in this family (unpublished data, see also Fig. 2f and ref. 3). The third family contains poliovirus type 2, and therefore all three serotypes of poliovirus<sup>6,12</sup>. The fourth family contains Coxsackie virus B3 and thus probably all or most of the other B Coxsackie virus<sup>7,12</sup>. Ad2 and probably at least several other adenoviruses are in this family<sup>2</sup>.

It is possible that receptor specificity influences virus tropism in tissues and organs, and thereby contributes to patterns of viral pathogenesis<sup>13,14</sup>. The finding that Coxsackie virus A21 ('Coe virus') shares receptors with a large number of human rhinoviruses may be related to its ability to produce cold-like symptoms<sup>15</sup>. Both B Coxsackie viruses<sup>1,16</sup> and adenoviruses<sup>17</sup> produce persistent infections in HeLa cells cultured in the presence of human serum. It will be important to determine if receptors for CB3 and Ad2, or CA21 and HRV-14 are covariant in different tissues or different host species.

Future work should attempt to determine the physiological and biochemical identity of the specific virus receptors, this could be rewarding because virus receptors have been used as markers in cytogenetic studies<sup>4,18</sup>.

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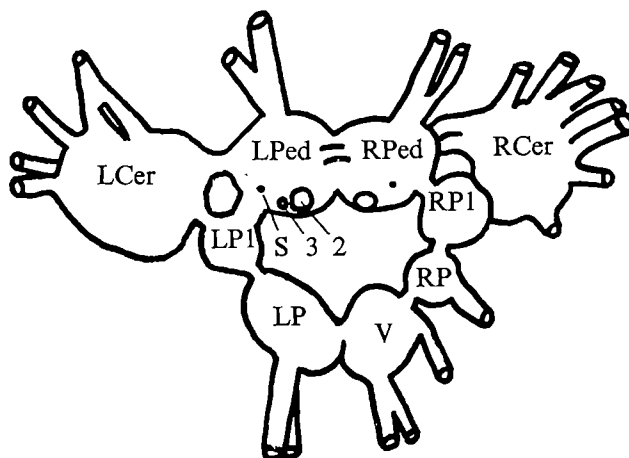
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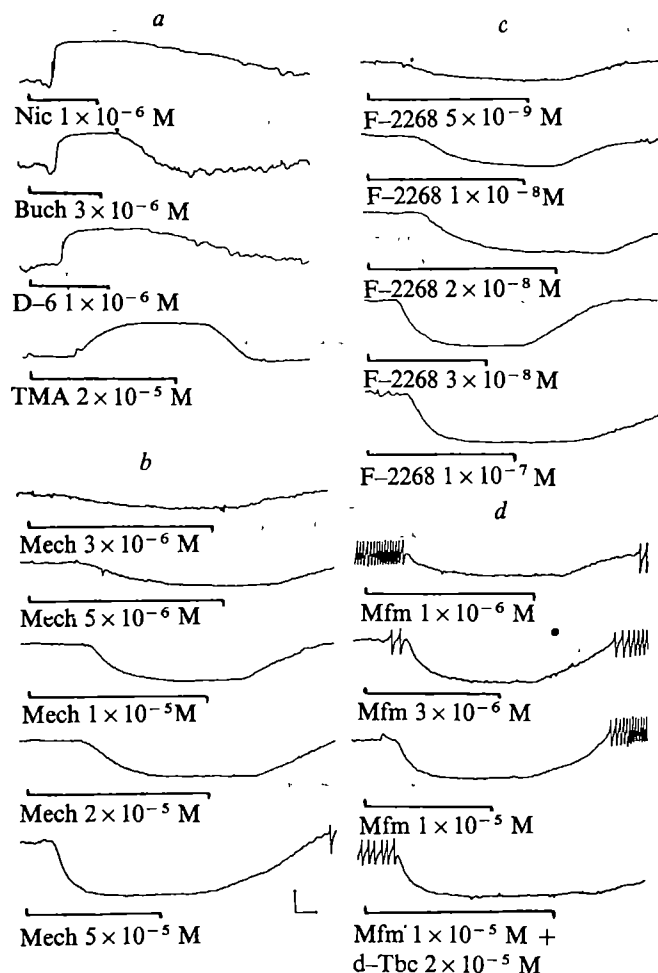
## Two kinds of cholinoreceptors on the membrane of the completely isolated identified *Planorbarius corneus* neurone

We have found that an identified neurone of the *Planorbarius corneus* left pedal ganglion (LPed-2 neurone, Fig. 1) responds with depolarisation to nicotinomimetics and with hyperpolarisation to some muscarinomimetics. The LPed-2 neurone of *P. corneus* contains dopamine<sup>1</sup> and is the dopaminergic interneurone sending depolarising and hyperpolarising impulses to some neurones of the visceral and the left parietal ganglion<sup>2</sup>. Acetylcholine (ACh) alone produces depolarisation like a nicotinomimetic; in the presence of tubocurarine, ACh hyperpolarised the cell membrane like a muscarinomimetic. The suggestion has been made that two kinds of cholinoreceptors (ChR) exist on the membrane of the LPed-2 neurone, differing in both the pharmacological properties and the ionic permeability changes they control<sup>3</sup>. Two different types of ChR have been described on the membrane of some identified *Aplysia* neurones<sup>4,5</sup>. Experiments<sup>3</sup> performed on the LPed-2 neurone in the ganglionic ring where it receives synaptic inputs from various interneurons which also can respond to ACh do not exclude the possibility that the two kinds of responses are attributable to the interference of some interneurons masking the actual effect of ACh on the LPed-2 neurone.

To resolve this problem we performed experiments on completely isolated LPed-2 and LPed-3 neurones (Fig. 1). The method described by Kostenko<sup>6</sup> was used. The ganglionic

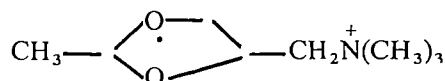
Fig. 1 Diagram of the dorsal surface of *P. corneus* circumoesophageal nerve ring. Cerebro-cerebral connective is cut to expose the pedal ganglia. Ganglia: LPed, left pedal; LCer, left cerebral; LPI, left pleural; LP, left parietal; V, visceral; RP, right parietal; RPI, right pleural; RCer, right cerebral; RPed, right pedal. LPed-2 (2) and LPed-3 (3) neurones can be easily identified: LPed-2 is the biggest of the left pedal ganglion<sup>1</sup>; LPed-3 can be identified by its position between LPed-2 and statocyst (S) and by the characteristic red colour of the surrounding glial cells.





circumoesophageal ring was treated with 0.3% Pronase solution for 30–40 min, then the sheaths were removed and single identified neurones were isolated using electrolytically sharpened tungsten needles. The isolated neurone was placed in a chamber (1.5 ml) perfused with saline solution (50 mM NaCl, 1.6 mM KCl

**Fig. 2** Effects of nicotinomimetics and muscarinomimetics on the completely isolated LPed-3 neurone. All records were obtained in the same experiment. The resting transmembrane potential was equal to  $-60$  mV. Drugs were added to the perfusion fluid at the upward arrow and washed out at the downward arrow. Calibration  $10$  s,  $10$  mV here and in Fig. 3 refers to de- and hyperpolarisation but not to the spikes. *a*, Depolarising responses to nicotinomimetics. Approximately equally effective concentrations were chosen. Nic, nicotine; BuCh, butyrylcholine; D-6, suberyldicholine<sup>17</sup>  $(\text{CH}_3)_3\text{N}-\text{CH}_2\text{CH}_2-\text{O}-\text{CO}-(\text{CH}_2)_6-\text{CO}-\text{O}-\text{CH}_2\text{CH}_2-\text{N}(\text{CH}_3)_3$ ; TMA, tetramethylammonium. *b-d*, Hyperpolarising responses to muscarinomimetics. *d*, Methylfurmethide (Mfm); note that at Mfm concentrations of  $10^{-8}$  M hyperpolarisation is greater in the presence of tubocurarine showing that Mfm activates at this concentration both kinds of ChR hyperpolarising response being partly masked by simultaneous depolarisation. *b*, Acetyl- $\beta$ -methylcholine (Mech). *c*, dioxolan F-2268.



Note that F-2268 is the most potent: its equeffective hyperpolarising doses are three orders of magnitude smaller than those of Mech or Mfm.

$4$  mM  $\text{CaCl}_2$ ,  $8$  mM  $\text{MgCl}_2$ , Tris-buffer  $0.25$  g l<sup>-1</sup>,  $\text{HCl} \leq \text{pH}$   $7.5$ ) and impaled with a microelectrode filled with  $2.5$  M KCl. Membrane potential was recorded using the standard intracellular technique<sup>7</sup>. Drugs were added to the perfusion fluid.

After isolation the LPed-2 neurone retains the rhythmic spontaneous activity which it characteristically displays in the ganglion. In the ganglion the LPed-3 neurone showed non-rhythmic discharges or permanent oscillations of transmembrane potential. After isolation it usually became silent or discharged rhythmically.

We found that LPed-2 and LPed-3 neurones in the isolated state are depolarised by nicotinomimetics (Fig. 2*a*) and hyperpolarised by muscarinomimetics (Fig. 2*b*, *c*, and *d*); this means that the isolated neurones respond in the same way as when they are *in situ* in the brain. The response to ACh is more complicated and depends on the concentration used (Fig. 3). Small ACh concentrations ( $5 \times 10^{-8}$  M– $2 \times 10^{-7}$  M) caused hyperpolarisation. With greater concentration the cell begins to repolarise in the presence of ACh; after washing out the hyperpolarisation reappears. This response suggests that ACh

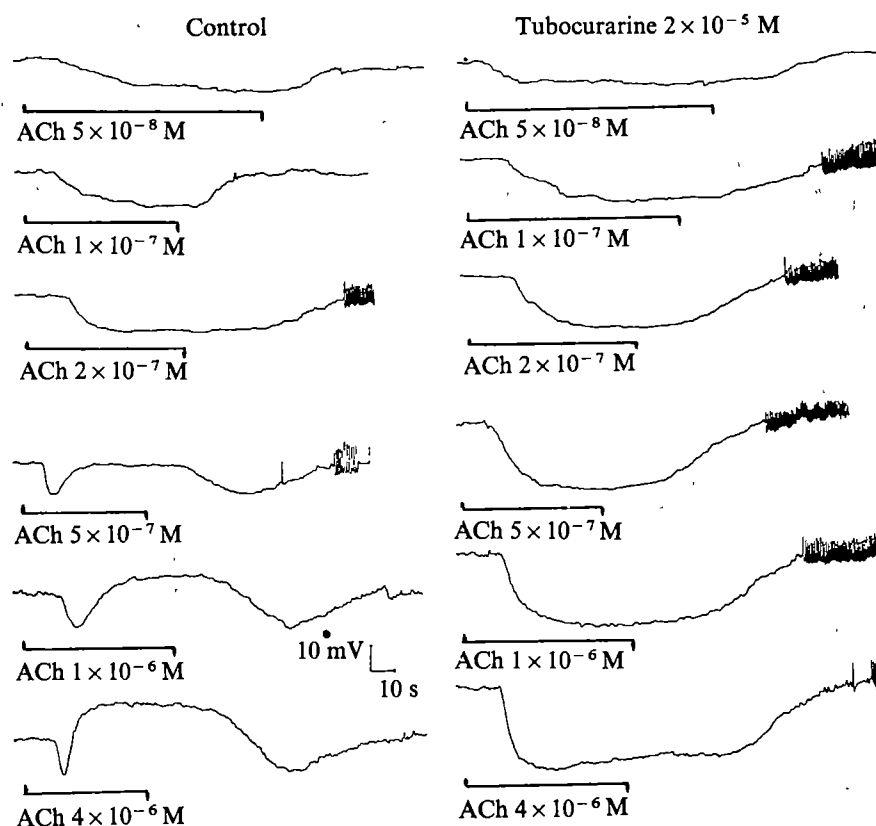
**Table 1** The ionic dependence of depolarising and hyperpolarising responses of the isolated LPed-3 neurone to cholinomimetics

Drug and concentration (mol)	<i>a</i> . Changes in the external chloride and sodium concentration							
	Responses (mV)				Responses (mV)			
	at different $[\text{Cl}^-]$ concentrations (mM)				at different $[\text{Na}^+]$ concentrations (mM)			
	$[\text{Cl}^-]$ 75*	$[\text{Cl}^-]$ 38	$[\text{SO}_4^{2-}]$ 38	$[\text{Cl}^-]$ 75*	$[\text{Cl}^-]$ 38	$[\text{C}_2\text{H}_5\text{COO}^-]$ 38	$[\text{Cl}^-]$ 75*	$[\text{Na}^+]$ 50*
ACh ( $1 \times 10^{-6}$ )	+11				+28		+11	
ACh ( $2 \times 10^{-6}$ )	+14				+20		+15	
ACh ( $1 \times 10^{-6}$ )	+7				+17			
ACh ( $5 \times 10^{-7}$ )	+11	+20		+10				
Nic ( $1 \times 10^{-6}$ )	+11	+16						
D-6† ( $1 \times 10^{-6}$ )	+11	+18			+19		+11	
F-2268 ( $1 \times 10^{-6}$ )	-13	-12						
F-2268 concentrations (Mol)	<i>b</i> . Changes in the external potassium concentration							
	Response (mV)				Response (mV)			
	External potassium concentrations (mM)				External potassium concentrations (mM)			
	1.6*	0.8	1.6*	1.6*	3.2	1.6*	1.6*	1.6*
$1 \times 10^{-8}$	-4	-9	-21	-23	-13	-21	-21	-21
$5 \times 10^{-8}$	-15	-23	-13	-19	-8	-14	-14	-14
$1 \times 10^{-7}$	-21	-28	-4	-21	-12	-17	-17	-17
$5 \times 10^{-8}$	-10	-20	-8	-19	-12	-17	-17	-17
$1 \times 10^{-7}$	-4	-11	-4	-19	-12	-17	-17	-17
$3 \times 10^{-7}$	-7	-17	-8	-21	-12	-17	-17	-17
$2 \times 10^{-8}$	-4	-11	-4	-19	-12	-17	-17	-17
$5 \times 10^{-8}$	-7	-17	-8	-21	-12	-17	-17	-17
$2 \times 10^{-7}$			-15	-15	-3	-15	-15	-15

\*Normal saline

†D-6 (suberyldicholine)<sup>17</sup> is a nicotinomimetic agent deprived of any muscarinic properties.

**Fig. 3** Effect of tubocurarine on the responses of the completely isolated LPed-3 neurone to ACh. All records were obtained in the same experiment. Note that with small ACh concentrations there is no difference between control responses (left column) and those in the presence of tubocurarine (right column); probably N-ChR are not excited by these ACh concentrations. At  $2 \times 10^{-7}$  M hyperpolarising response is greater in the presence of tubocurarine. Higher ACh concentrations caused a two-component potential change in the control but pure hyperpolarisation in the presence of tubocurarine (with the exception of response to ACh ( $4 \times 10^{-6}$  M) where a slight repolarisation can be seen, suggesting that the tubocurarine concentration ( $2 \times 10^{-5}$  M) is not high enough to protect the nicotinic ChR against ACh concentration as high as  $4 \times 10^{-6}$  M. The resting potential was equal to 43 mV. Tubocurarine ( $2 \times 10^{-5}$  M) was added to the perfusion fluid 2 min before the addition (upward arrow) of the solution containing both ACh and tubocurarine. Calibration 10 mV, 10 s.



activates two kinds of ChR, with those mediating hyperpolarisation being sensitive to lower ACh concentration. Nicotinic ChR (N-ChR) of *P. corneus* and *Limnaea stagnalis* neurones are blocked by tubocurarine<sup>8,9</sup> as are those of *Aplysia* neurones<sup>10</sup>. We observed that in the presence of tubocurarine ACh caused pure hyperpolarisation, as do muscarinomimetics (Fig. 3). These results indicate that two kinds of ChR coexist on the membrane of the completely isolated neurones, LPed-2 and LPed-3, with the isolated cells much more sensitive to ACh and cholinomimetics (especially muscarinomimetics) than non-isolated neurones.

Of the two types of ChR found on the membranes of the isolated neurones those mediating depolarisation are similar to N-ChR in vertebrates: they are excited by all nicotinomimetics and are blocked by tubocurarine. Receptors mediating hyperpolarisation seem to resemble the muscarinic ChR (M-ChR) of vertebrates less closely. We have studied the hyperpolarising potency of a series of drugs known to excite M-ChR: dioxolan F-2268 (ref. 11); methylfurmethide, acetyl- $\beta$ -methylcholine; oxotremorine; arecoline; carbacholine; pentyltrimethylammonium. All of those drugs (with the exception of oxotremorine) caused hyperpolarisation. The most potent proved to be F-2268 ( $EC_{50} = 2 \times 10^{-8}$  M) which is very active on vertebrate M-ChR (refs 11 and 12). Other muscarinomimetics—even those known to have a high potency on vertebrate M-ChR (such as methylfurmethide and oxotremorine)—were far less potent than F-2268 in producing mollusc neurone hyperpolarisation. For example, methylfurmethide proved to be 350 times as weak as F-2268. Oxotremorine produced no hyperpolarisation even in high concentration. It seems, therefore, that mollusc neurone ChRs mediating hyperpolarisation differ in their chemical sensitivity from vertebrate M-ChR.

The difference becomes even more evident when studying the potency of typical muscarinolytics. Neither atropine nor scopolamine can prevent the hyperpolarisation caused by ACh or by muscarinomimetics. The alkylating agent benzilylcholine mustard (A-5 ref. 13), known to block selectively and irreversibly vertebrate M-ChR (refs 14 and 15), was found to block

neurone hyperpolarisation selectively and irreversibly as well, without changing the depolarising (nicotinic) response. The concentration needed ( $2 \times 10^{-4}$  M for 15 min) was, however, five orders of magnitude greater than that needed to block M-ChR in the guinea pig ileum. Like the slow hyperpolarisation of *Aplysia* neurones<sup>5</sup>, the hyperpolarisation of LPed-2 and LPed-3 neurones can be prevented by tetraethylammonium ( $5 \times 10^{-5}$  M).

The study of the ionic basis of the depolarising and hyperpolarising responses are in progress; Table 1 shows the preliminary results. We have found (Table 1) that the replacement of 38 mM (one half) of the chloride ions in physiological solution by 38 mM of sulphate or propionate ions increases the depolarising responses to ACh, nicotine and the nicotinomimetic agent suberyldicholine (D-6), (ref. 16). The replacement of one half (25 mM) of sodium chloride in physiological solution by an equal concentration of lithium chloride did not change the depolarising response to D-6. These results suggest a chloride dependence of the depolarising response to ACh and to nicotinomimetics. As we used microelectrodes filled with KCl it can be concluded that the depolarising response to ACh and nicotinomimetics is attributable to the increase of the intracellular chloride concentration (as a result of the diffusion of chloride from the microelectrode) which in turn leads to the change in the chloride equilibrium potential and finally to the change in the sign of response. To check this possibility the microelectrodes filled with potassium citrate (1 M),  $K_2SO_4$  (0.5 M) and  $Na_2SO_4$  (1 M) were used in several experiments. Nicotinomimetic agents still caused depolarisation with these microelectrodes and there was a depolarising phase in the response to ACh.

No change in the hyperpolarising effect of F-2268 was observed when the chloride concentration was halved (Table 1). Halving (doubling) the potassium concentration increased (decreased) the hyperpolarising response to F-2268 (Table 1b). Thus this response seems to be potassium dependent.

Two kinds of ChR found on completely isolated *P. corneus* neurones seem to closely resemble those on the neurones of



the *Aplysia* pleural ganglion<sup>6</sup> in both pharmacology and ionic dependence.

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## Fluorescence changes during electrical activity in frog muscle stained with merocyanine

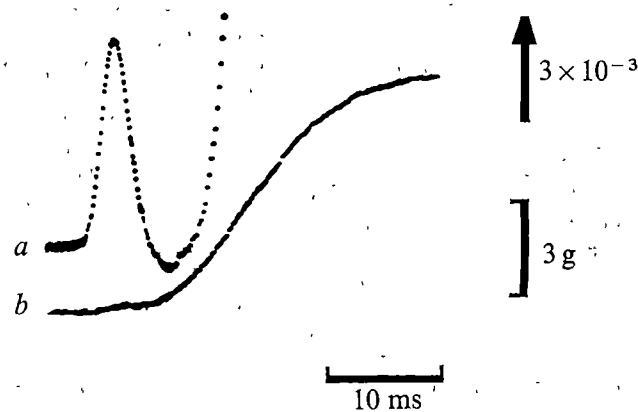
NERVE membranes stained with various fluorescent dyes show changes in fluorescence intensity concomitant with changes in transmembrane potential<sup>1–4</sup>. Cohen *et al.*<sup>4</sup> described a number of dyes that exhibit changes in fluorescence emission which vary linearly with transmembrane potential. Two of these have been used to investigate the excitation-contraction process in skeletal muscle<sup>5–7</sup>. Whole muscles and single fibres stained with Nile Blue A were reported to give fluorescence intensity changes on stimulation arising from the membranes of the sarcoplasmic reticulum<sup>5,6</sup>. Muscles and fibres stained with Merocyanine 540 on the other hand, give very early changes in fluorescence intensity when stimulated electrically<sup>8,7</sup>. Landowne<sup>7</sup> proposed that since the merocyanine signal disappeared after detubulation in snake costocutaneous muscles, the fluorescence change must arise from the T-system membranes.

We describe here experiments in which the extrinsic fluorescence signal is compared with the membrane potential in bundles of frog skeletal muscle fibres stained with Merocyanine 540. Our results partially agree with Landowne's conclusion that this dye monitors electrical activity in the T-system membranes; they do indicate, however, that the surface membrane also contributes to the fluorescence signal. The proportion of the contributions from both membrane systems can be roughly estimated as 9:1 (T-system-surface membrane) but important assumptions underlying this value must be investigated in detail.

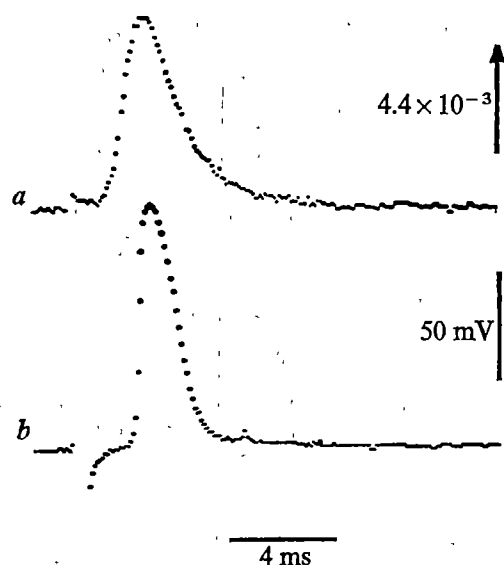
Bundles of 40–200 fibres from the sartorius and the semitendinosus muscles of the Chilean frog, *Calyptocephalella gayi* were used instead of single fibres to extend the life of the preparation exposed to the toxic effects produced by the merocyanine dye in the presence of light and oxygen (see below and ref. 4). They were dissected and mounted horizontally in the narrow trough of a lucite chamber. The trough was separated into three compartments with Vaseline. The central compartment (4 mm wide) had one

of the walls and the bottom made of glass to allow light transmission. The other wall was a platinised Pt plate used to pass current through the muscle fibres to the lateral compartments. The bundles were stimulated in two different ways; mode A, by passing current between the central compartment and the lateral ones to give simultaneous polarisation of the fibres in the central compartment; and mode B, by passing current between two Pt plates in one of the lateral compartments to propagate action potentials travelling to the central compartment. The bundle was kept in place by connecting one tendon to a strain gauge transducer and the other to forceps. The chamber was placed on an optical bench so that the light coming from a tungsten-halogen lamp could be filtered and focused on the bundle in the central compartment of the chamber. At 90° with respect to the illuminating beam, the light was filtered and collected by a photodiode connected to the recording apparatus (for details see legend of Fig. 1 and ref. 5). As fibres stained with Merocyanine 540 were damaged by exposure to light in the presence of oxygen in the bathing medium, the illumination periods were minimised and nitrogen was bubbled in the bathing solution between experimental runs. Nevertheless, after several periods of illumination, action potentials became wider and the fibres eventually lost their excitability.

Figure 1 shows the relationship between the fluorescence signal (trace a) and tension development (trace b) in a bundle of about 40 sartorius fibres stimulated by a single-current pulse in mode B. The bundle had been exposed to the staining solution for 45 min and then transferred to Ringer's solution for recording. Figure 1a shows a transient fluorescence signal followed by a movement artefact when tension starts to develop (trace b). To eliminate this artefact, and to observe the recovery phase of the early



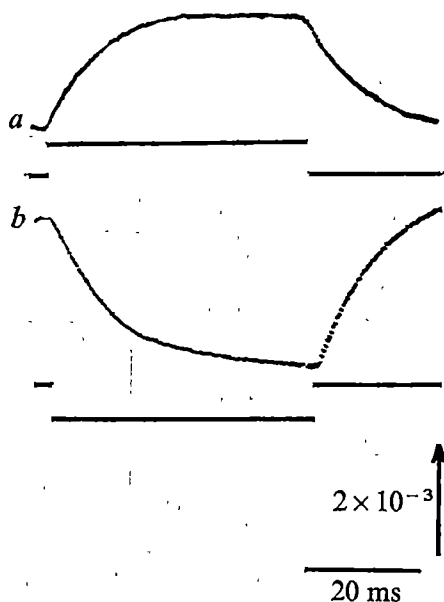
**Fig. 1** Merocyanine fluorescence signal (a) and tension (b). The change in fluorescence is expressed as the ratio of change in light intensity to resting fluorescence light intensity. An increment in fluorescence intensity is indicated by the arrow. Temperature 21 °C. In all experiments a Baird-Atomic interference filter (35-15-16) with a central wavelength of 548.6 nm was used as a primary filter to render the incident light quasi-monochromatic. The light from the bundle was filtered by a barrier filter (Schott RG-610) which absorbed wavelengths shorter than 610 nm. This was used to filter the light emitted at 90° with respect to the source and was placed beneath the bottom of the central compartment of the chamber. Below the secondary filter, the light was collected by a photodiode (PV-444, E. G. & G.) followed by a current-voltage converter. The electrical signals from the photodiode were filtered by a bandpass filter (time constant 0.2 ms), amplified by a Tektronix 3A3 amplifier and then fed with either the membrane potential from a microelectrode amplifier or the tension signal from the transducer into a digital machine which will be described elsewhere. This machine enabled us to average the signal and to record the output using a standard high-fidelity tape recorder or chart recorder. The Ringer's solution contained 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl<sub>2</sub>, 2.15 mM Na<sub>2</sub>HPO<sub>4</sub> and 0.85 mM NaH<sub>2</sub>PO<sub>4</sub>, pH = 7.1 (ref. 8). The staining solution was Ringer's plus 0.05 mg ml<sup>-1</sup> Merocyanine 540 (Eastman Kodak), Pluronic F-127 0.02% (BASF Wyandotte) and 1% ethanol.



**Fig. 2** Fluorescence signal and action potential. *a*, A single sweep displaying the change in fluorescence intensity on stimulation of a bundle of more than 100 fibres from the semitendinosus muscle. *b*, Action potential recorded by a microelectrode impaled in a fibre with a resting potential of  $-86$  mV in the centre of the illuminated region of the central compartment. Both records were taken simultaneously for a single stimulus of  $0.2$ -ms duration in the depolarising direction. Capacitive pick-up by the microelectrode distorted the membrane potential record at short times after the stimulus pulse was given. The bundle was stretched to  $1.4$  times its slack length and was immersed in  $345$  mM NaCl hypertonic Ringer's solution (see text). The tension record was flat. Temperature  $22^{\circ}\text{C}$ .

signal it was necessary to block development of tension. This was achieved by stretching the bundles to between  $1.3$  and  $1.5$  times their slack lengths and immersing them in hypertonic solutions with  $230$  mM NaCl added to Ringer's solution<sup>5</sup>. Figure 2 shows the results of an experiment in those conditions. The bundle was stimulated with a  $0.2$ -ms

**Fig. 3** Fluorescence intensity changes with prolonged stimulation. *a*, Fluorescence intensity change during a depolarising current pulse after  $10^{-6}$  M TTX was added to the Ringer's solution. *b*, The resulting fluorescence signal during a hyperpolarising pulse of equal amplitude. Both records are the average of 20 sweeps. The time courses of the stimulus pulses are shown below each trace. The bundle was stretched to about  $1.5$  times its slack length and it was immersed in hypertonic Ringer's to prevent movement during depolarisation. Temperature  $20^{\circ}\text{C}$ .

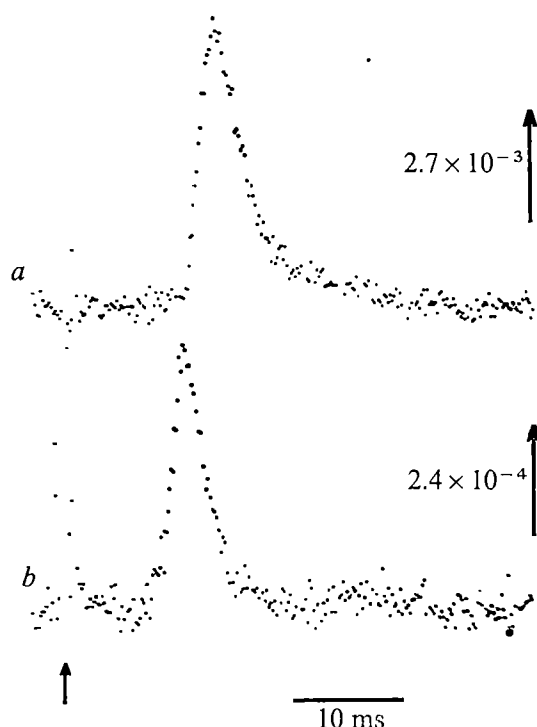


depolarising current pulse in mode A. The complete time course of the transient fluorescence signal is displayed, without the movement artefact, in trace *a*. Trace *b* is the simultaneous record of an action potential obtained by impaling a single fibre from the bundle with a microelectrode positioned in the centre of the illuminated region. The rising phase of the action potential ( $t_{1/2}=0.5$  ms) is steeper than the rising phase of the fluorescence signal ( $t_{1/2}=1$  ms) and the recovery phase of the latter ( $t_{1/2}=1.2$  ms) is slower than the repolarisation of the action potential ( $t_{1/2}=0.95$  ms). One reason why records 2*a* and *b* are different could be that the fluorescence signal is recorded simultaneously from a population of fibres of the bundle. The non-synchronous activation, due to different thresholds among fibres of the bundle, would be seen as an apparently slower signal. This possibility was tested by impaling several fibres in the illuminated region and different delays were found between the peak of the action potential and the peak of the fluorescence signal. In the experiment shown in Fig. 2, the impalement of five fibres showed that the peak of the fluorescence signal preceded, or was delayed with respect to the peak of the action potential within a range of  $0.85$  ms. This range would explain the differences on the rising phase half times between the fluorescence signal and the action potential. This argument, however, is less convincing in explaining the differences in the falling phase half times because the repolarisation of the action potential is a slower process. An alternative, but not exclusive, explanation for the differences between the action potential and the light signal time courses would be that the latter is monitoring the slower potential changes of the T-system membranes<sup>9</sup>. In fact, the merocyanine fluorescence signal recorded from a single fibre by Oetliker *et al.*<sup>9</sup> has a similar time course to that shown in Fig. 2*a*.

Figure 3 shows the fluorescence signals recorded in a bundle after addition of  $10^{-6}$  M tetrodotoxin (TTX) to the external solution, when  $45$ -ms hyperpolarising (Fig. 3*a*) and depolarising (Fig. 3*b*) pulses were applied in mode A. The characteristic 'creep', produced by the anomalous rectifier, is observed in Fig. 3*b*. The time constant of the membrane as monitored by the dye was calculated from these and three more experiments to be  $17.9 \pm 1.22$  (s.e.) ms ( $n=7$ ). This value is close to those measured with microelectrodes<sup>12,13</sup>. If most of the fluorescence signal is coming from the T-system membranes, the optical time constant should be slightly longer than the electrical one. This difference, however, might be within experimental error in our measurements.

To obtain more information about the membrane systems monitored by the merocyanine dye, we carried out a series of detubulation experiments using the procedure described by Eisenberg *et al.*<sup>10</sup>. In this method the bundle is transferred from the glycerol-containing solution to Ringer's containing  $5$  mM Ca and  $5$  mM Mg which maintains the resting potential at around  $-82$  mV (ref. 15). Figure 4*a* corresponds to a propagated fluorescence signal before detubulation of a bundle of less than  $60$  fibres immersed in hypertonic solution and stimulated in mode B. The bundle was stimulated  $19$  mm away from the illuminated region and the propagated signal was detected about  $11.3$  ms after the stimulus pulse. The speed of propagation of the signal can be roughly calculated to be  $168$  cm s<sup>-1</sup>, which agrees with previous measurements of action potentials speed<sup>14</sup>. After detubulation, the fluorescence signal changed (Fig. 4*b*). The amplitude of the signal is reduced by a factor of ten and the propagation velocity increased to  $229$  cm s<sup>-1</sup>,  $36\%$  faster than before detubulation. The decay phase of the light signal in the detubulated bundle (Fig. 4*b*) is faster than that shown in Fig. 4*a*.

The fluorescence signal was used in two experiments to measure the time constant of the membrane, obtained with long pulses applied to TTX-treated bundles. The average



**Fig. 4** Propagated fluorescence signals before and after detubulation. *a* and *b* were obtained from the same bundle of 60 semitendinosus fibres in 345 NaCl hypertonic Ringer's solution and stretched to about 1.5 times its slack length. *a*, A single sweep before detubulation; *b*, average of 40 sweeps after detubulation. The arrow indicates the time the stimulus pulse was applied. Pluronic 127 was not used in the staining solution. Temperature 20 °C.

value was  $7.33 \pm 0.79$  (s.e.) ms ( $n=6$ ), significantly faster than in intact bundles. No significant 'creep' was observed during hyperpolarising pulses. These results demonstrate that detubulation has a similar effect on the fluorescence signal and the membrane potential recorded with microelectrodes. The amplitude of the fluorescence signal, however, is reduced by the glycerol treatment, but the action potential amplitude is virtually unaffected<sup>16</sup>. If we assume that the magnitude of the signal is proportional to the amount of membrane stained, we estimated that  $\sim 10\%$  of the total membrane is not affected by detubulation. In other experiments this value ranged from 4% to 20%. It could be argued that the 90% decrease of the light signal is produced by damage during detubulation. Apart from evidence that the glycerol treatment, using raised Ca and Mg concentrations, gives a better survival of the fibres<sup>10,15</sup>, there are several observations which make this explanation unlikely. First, the increased speed of the falling phase of the fluorescence signal after detubulation is consistent with the idea that Merocyanine 540 monitors the electrical activity of both surface and T-system membranes in intact fibres, but predominantly surface membrane in detubulated fibres. Second, the faster time constant of the light signal in response to long pulses in TTX-treated fibres after detubulation is also compatible with the loss of T-system membrane capacity—which is predominant in intact fibres and there produces a longer time constant for the light signal. Finally, in the experiments performed in normal Ringer's, tension was reduced by detubulation to a lower proportion (0.4–2.2%) than the light signal amplitude (4–20%), indicating that most of the remaining fluorescence signal came from fibres that were inactivated mechanically; this in turn, indicates that the signal originated partially in the surface membrane. More detubulation experiments will be required to study in detail the relative contribution of the surface and tubular membranes and the qualitative changes in the

time course of the fluorescence signal before and after detubulation.

Merocyanine 540 very probably does not penetrate to the interior of the fibres because a larger and slower signal would be expected from the sarcoplasmic reticulum, like that seen with the lipid-soluble dye Nile Blue A<sup>5,6</sup>. Also, recent experiments in squid axon microinjected with Merocyanine 540 suggest that the dye, with its fixed negative charge, does not cross the neuronal membrane rapidly (B. M. Salzberg, personal communication).

The results presented here may prove useful for those investigations in which monitoring membrane potentials from a population of muscle fibres is required. They complement information obtained with other dyes<sup>5,6</sup> that monitor membrane potential changes in the sarcoplasmic reticulum. Using Merocyanine 540 and Nile Blue A it is now possible to study separately the potential changes across different membrane systems, some of them inaccessible to microelectrodes and intimately involved in the chain of events of the excitation–contraction coupling in skeletal muscle.

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## Effect of temperature and pressure on polymerisation equilibrium of neuronal microtubules

MICROTUBULES are fibres, sometimes many micrometres long, present in all eukaryotic cells, formed by the reversible polymerisation of the protein tubulin. These fibres not only have a structural role, but the process of their assembly and disassembly itself controls several cell functions<sup>1</sup>.

We have investigated the effect of temperature and pressure on this process, and show here that their main influence is on the nucleation equilibrium. With this reanalysis of the data, we make some speculations about some of the molecular processes involved.

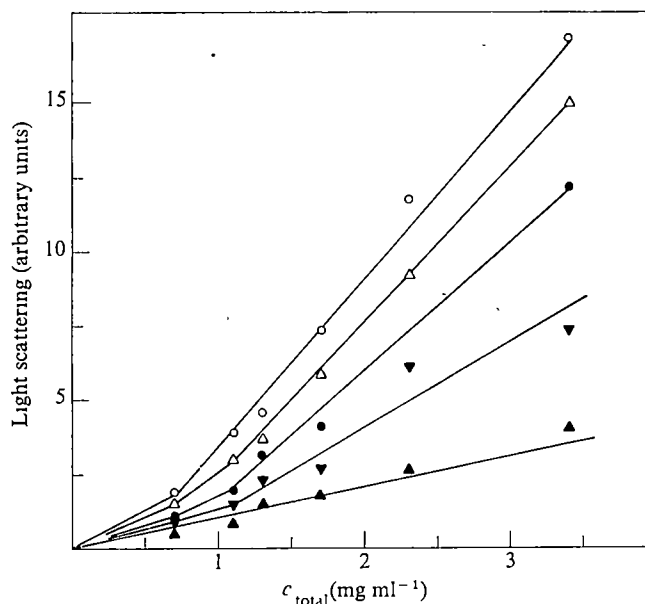
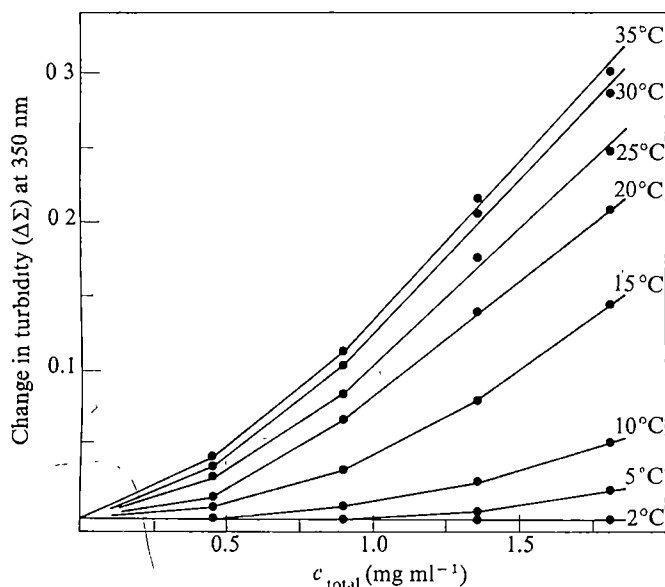
No detailed molecular model of the reaction is yet available. Depolymerisation, either by adding calcium or cooling to 4 °C, gives a mixture of a 6S protomer (an  $\alpha\beta$ -dimer of total molecular weight 120,000) and a 36S fraction<sup>2</sup>. This 36S protein is a ring or spiral-like structure of  $\sim 23$  protomers, formed in the presence of the 'τ factor'<sup>3</sup>. Copolymerisation of the 6S and 36S fractions leads to the formation of the microtubules.

Until now equilibrium data for the polymerisation have always been analysed in terms of a propagation equilibrium constant, the reciprocal of the critical concentration. In this way Gaskin *et al.*<sup>4</sup> were able to calculate an apparent reaction enthalpy change for propagation of 21 kcalorie mol<sup>-1</sup>, for temperatures < 25 °C, and the corresponding entropy change of 100 EU. At temperatures between 25 and 37 °C the enthalpy change is essentially zero and the entropy change is ~ 10 EU. The effect of pressure has been analysed<sup>5,6</sup>, using the same assumption, at a single protein concentration. It was found that propagation is accompanied with a volume change of 90 ml mol<sup>-1</sup>.

Essential in all these analyses is the assumption that the protomer concentration remains constant from the critical protein concentration on. This is, however, true only in the limits of very high protein concentration, and not in the range usually studied, as shown by ultracentrifugation<sup>7</sup> and by the data of Fig. 1. Here the change of turbidity at 350 nm is plotted as a function of total protein concentration, at different temperatures. The reversibility was checked in separate experiments. The data clearly show that the protomer concentration is not constant, both because the concentration range studied is rather low, and because an additional equilibrium, the nucleation equilibrium, determines the protomer distribution.

The effect of pressure was analysed at 35 and 15 °C, by measuring light scattered at 90 °C, as described earlier<sup>8</sup>. Experiments were done in conditions of complete reversibility which was maintained up to 1,000 atm at 35 °C. The increase in pH of the MES buffer with increasing pressure was very small and so was neglected. The results are given in Fig. 2, which shows that the nucleation equilibrium is again important. The results of Fig. 2 also show that there is a link between the effect of pressure and temperature. At 15 °C, 500 atm is sufficient to produce complete dissociation, while at 35 °C only a small pressure effect is observed. A link between the effect of pressure and temperature is also found for a number of other proteins, such as flagellin<sup>9</sup> and glutamate dehydrogenase<sup>10</sup>, although the effect is much less pronounced for these. O'Connor *et al.*<sup>11</sup> did not find a measurable pressure effect at 37 °C and 700 atm, and

**Fig. 1** The effect of temperature on the turbidity of microtubules at different total concentrations. Rat brain tubulin was prepared according to Shelanski<sup>16</sup>. After the third polymerisation cycle it was dialysed against MES buffer at pH 6.4, with the following composition: 50 mM MES, 70 mM KCl, 1 mM EGTA and 0.5 mM MgCl<sub>2</sub>. After dialysis GTP was added up to 1 mM. Protein concentration was determined with the method of Lowry<sup>17</sup>. The quality of the microtubules was checked by electron microscopy.



**Fig. 2** The effect of pressure on light scattering by microtubules at different total concentrations. The same buffer as in Fig. 1 was used. The accuracy of the measurements is much less than in Fig. 1, because all the measurements had to be done on samples from different preparations. For the sake of clarity only a limited number of data are given. The measurements are corrected for window effects and lowering of the incident light intensity as a consequence of turbidity. ○, data at 1 atm at 35 °C; △, 500 atm at 35 °C. ●, ▼, ▲, for 1, 200, 500 atm respectively at 15 °C.

they claim that neurotubules are therefore different from other microtubules on the basis of a different pressure sensitivity, without taking into account the effect of the temperature. At 20 °C, where microtubules of non-neuronal origin are usually studied, neurotubules also show a large pressure dependence.

We have attempted to analyse these data quantitatively in terms of an equilibrium constant for nucleation and propagation. The system behaves completely reversibly in the presence of an excess of GTP, whose role is not, however, clear. The thermodynamic parameters obtained are therefore equilibrium parameters for the polymerisation coupled to GTP-hydrolysis, or activation parameters in the case of an irreversible consumption of GTP and the development of stationary states.

We assume that there is a nucleation step and that growth only occurs at the nuclei or tube ends (see ref. 13). (In fact Dentler *et al.*<sup>14</sup> show that growth occurs only at one end of the tube.) The relationship between  $c_p$ , the number concentration of protomers present in polymers, and  $c_1$  the protomer concentration is then:

$$c_p = Ac_1/(1-Kc_1)^2 \quad (1)$$

Here  $K$  is the propagation equilibrium constant, which is assumed to be independent of the degree of polymerisation ( $i$ ) for  $i > n$ , where  $n$  is the number of protomers involved in the nucleus.  $AK^{n-1}$  gives the nucleation equilibrium constant, with  $A$  a dimensionless equilibrium constant that corresponds to the destabilisation of the nucleus with respect to an  $n$ -mer in the middle of the polymer. This equation does not necessarily give the right distribution for the intermediate stages between protomers and nucleus. The contribution of these terms is, however, negligible. Equation (1) can then be rearranged

$$(c_1/c_p)^{1/2} = A^{-1/2}(1-Kc_1) \quad (2)$$

and the data are analysed accordingly.

Gaskin *et al.*<sup>4</sup> showed that light (wavelength  $\lambda$ ) scattered by microtubule solutions is nearly independent of the length of the tubules which can be proved directly by sonication of the



tubules, and also by the dependence of the scattering on  $\lambda^{-3}$ . We found that this dependence is maintained down to 8 °C, while at 4 °C a  $\lambda^{-4}$  dependence was found. This indicates that light scattering is indeed a good means of measuring  $c_p$  even at the lowest degrees of polymerisation.

Assuming that the steepest slope in Fig. 1 corresponds to the specific turbidity of the polymers, as checked by ultracentrifugation,  $c_1$  and  $c_p$  can easily be calculated. As in previous treatments<sup>4,6</sup>  $c_1$  is defined as the total number concentration of protomers, regardless of their distribution between 6S and 36S forms. Figure 3 shows that equation (2) fits the data well. The parameters  $A$  and  $K$  can be calculated simply and their logarithm plotted against  $T^{-1}$ , as shown in Fig. 4a. These data show that in the range of 25–10 °C the enthalpy and entropy changes are much larger for the parameter  $A$  than for the propagation equilibrium constant. In this case the theory predicts that at low temperatures, where the nucleus is destabilised, a small number of very long tubules will be present. This is confirmed both by the inverse third power dependence on the wavelength and by electron microscopy.

In the range of 35–30 °C smaller values for the thermodynamic parameters are found (see ref. 4). This change may be caused by a conformational transition of the protein, although the presence of a small amount of inactive protomer would cause a deviation in the low values of  $(c_1/c_p)^{1/2}$ .

The influence of pressure can be analysed similarly. Because of the limited accuracy of the measurements, however, a whole range of parameters can give a good fit to the data of Fig. 2. The logarithm of the extremes of these values are plotted against  $P$  in Fig. 4b. Although it is not possible to calculate accurate values for the volume changes, it is clear that the pressure sensitivity is larger at 15 °C, and this is more pronounced for the parameter  $A$  than for the propagation equilibrium constant. This may again be explained by a conformational transition between the two temperature ranges.

These results give some indication of the type of bonds involved. The small pressure dependence at temperatures between 25 and 35 °C is probably the consequence of the dominance of hydrophobic interactions, which are usually associated with small volume changes; large volume changes are generally found in ionic interactions<sup>15</sup>. It is thus possible that lowering the temperature decreases the stability of hydrophobic interactions, and changes the conformation which leads to the exposure of charged groups. Independent evidence for this conformational change comes from the large difference in affinity of colchicine for the protomers, at 4 and 35 °C and from circular dichroism studies<sup>18</sup>. This conformational change seems

Fig. 3 Linearisation of the data of Fig. 1 using equation (2). ▼, -10 °C; □, -15 °C; ■, -20 °C; ○, -25 °C; ▲, -30 °C and ●, -35 °C.

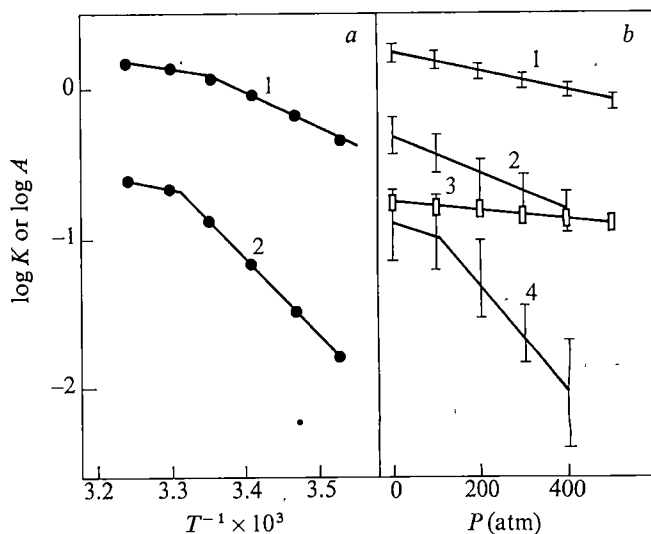
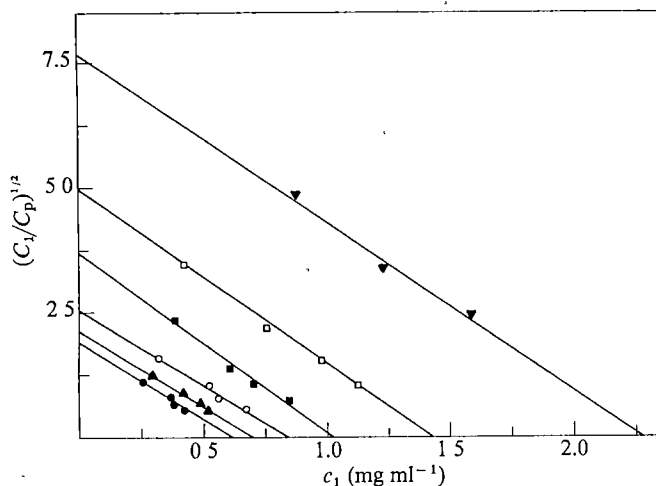


Fig. 4 a, Temperature dependence of the equilibrium constants. (1) For the propagation equilibrium constant  $K$  a value of  $\Delta H = 10$  kcalorie  $\text{mol}^{-1}$  and  $\Delta S = 34$  EU was found in the temperature range 25–10 °C. In the temperature range 35–30 °C, the values are halved. (2) For the nucleation parameter  $A$  the following values were obtained:  $\Delta H = 25$  kcalorie  $\text{mol}^{-1}$  and  $\Delta S = 80$  EU in the range 25–10 °C. At higher temperatures the values are about one-fifth of these. b, Pressure dependence of the equilibrium constants. Log  $K$  was plotted against  $P$  at 35 °C (1) and at 15 °C (2). The associated volume changes are 26 and 50  $\text{ml mol}^{-1}$  respectively. Log  $A$  was plotted against  $P$  at 35 °C (3) and at 15 °C (4). The estimated volume changes are 12 and 120  $\text{ml mol}^{-1}$  respectively.

to have its largest influence on the process of nucleation. It remains to be investigated whether other factors also exert their main influence on the nucleation equilibrium. This seems attractive, as a number of *in vivo* observations may be explained by the control of the nucleation process and the stability of the tube ends.

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## Androgen-sensitive midbrain sites and visual attention in chicks

NEUROANATOMICAL networks within the preoptic-hypothalamic continuum of the avian brain contain neurones that are androgen-sensitive<sup>1</sup> and involved in the control of precocial copulation<sup>2</sup>. In autoradiographic studies of the male chick brain, one of us (C.C.M.) has found midbrain structures, nucleus intercollicularis (ICo) and nucleus isthmo-opticus (IO) that concentrate radioactive testosterone or its metabolites. Neither of these structures has been implicated in the expression of sexual behaviour. Rather, there is evidence that both are involved in visual responsiveness that is not specifically associated with sexual behaviour. We wish to present the autoradiographic findings and relate them to the existing evidence of the functions of these two nuclei and testosterone in visual attention.

The technique has been described in detail elsewhere<sup>3</sup>. Male chicks were assigned to either an isolation ( $n=3$ ) or isolation and testosterone primer ( $n=3$ ) group. In the latter chicks 20 mg of testosterone propionate was subcutaneously injected on day 6 after hatching. On either day 15, 16 or 17 after hatching, male chicks received 200  $\mu$ Ci (1.3  $\mu$ g) of 1,2-<sup>3</sup>H-testosterone subcutaneously. Animals were decapitated 2 h after injection, their brains were removed, frozen in powdered CO<sub>2</sub>, sectioned in a microtome-cryostat, and the sections were mounted on slides precoated with photographic emulsion. After exposure for 121-161 d, the autoradiograms were developed and stained with thionin. Quantitative analyses of reduced silver grains beneath neurones were conducted by reviewing 50 cell bodies for each neuroanatomical area surveyed in every animal. Results, which will be published in full elsewhere, showed high mean concentrations for silver grains in neurones of the ICo, both in isolated ( $\bar{x}=13.77 \pm 1.26$  s.e.m.) and isolated and testosterone primed ( $\bar{x}=10.09 \pm 1.07$  s.e.m.) subjects, which appear to be even greater than in the nucleus praepopticus paraventricularis magnocellularis ( $\bar{x}=9.36 \pm 0.82$  s.e.m. isolates;  $\bar{x}=6.68 \pm 0.73$  s.e.m., isolates and testosterone primer). This medial preoptic nucleus is known to contain androgen-sensitive neurones and to be involved in the development of precocial copulation in male chicks<sup>1,2</sup>. In some animals autoradiograms extend into the posterior midbrain where the concentration of silver grains indicated a relatively high uptake of radioactive testosterone in the IO. Qualitative surveys for other midbrain areas, such as the tectum, nucleus mesencephalicus lateralis pars dorsalis, and central mesencephalic grey, did not show similar patterns of testosterone uptake. Control subjects receiving cold testosterone averaged no more than 1.80 grains per neurone in any brain region, and slides fogged with light before exposure showed no fading of the latent image. Although autoradiographic data from the songbird *Fringilla coelebs*<sup>4</sup> also indicate that ICo is androgen sensitive, no other report has established that the IO contains androgen-sensitive cells.

Andrew reviewed studies of the ICo<sup>5</sup>. He points out that electrical stimulation of this nucleus induces calling and

alerting in chicks while lesions produce muteness and impaired visual targetting behaviour, including loss of scanning behaviour in an open field, and a reduction of inspection and absence of exploratory pecking of moving targets and 'novel objects'. Andrew and de Lanerolle<sup>6</sup> have summarised these effects by saying that the lesioned chick "... ceases to treat visual (and probably other) stimuli as if they were conspicuous or highly valent". In previous studies Andrew<sup>7</sup> found that testosterone injections in chicks produced, in addition to precocial copulation, an increase in persistence in attending to objects. His subsequent work<sup>8</sup> has established that testosterone increases the probability of sustained visual attention to a particular stimulus or type of stimulus in competition with other distracting stimulation. In fact the effect seems to be the reverse of that produced by lesions of ICo so that the injected chick calls and orients to stimuli as if they were unusually conspicuous or valent. The facilitation of calling associated with the uptake of hormone by ICo may be part of the general attentional syndrome rather than the primary effect proposed by Zigmond *et al.*<sup>4</sup>, for Andrew<sup>10</sup> has made the point that all chick calls may belong to a general set of attention behaviours expressing an attentional state. We suggest, therefore, that the ICo is part of a neural system for facilitating orientation behaviour. Such a system has been suggested by Salzen and Parker<sup>11</sup> to arise in the archistriatum which is responsible for facilitating the subtelencephalic systems that execute head and body orientations to objects in response to decisional commands from other striatal regions. Certainly ICo receives fibres from the archistriatum through the tractus occipitomesencephalicus. Andrew<sup>12</sup> has also outlined an archistriatal source of descending influence on the ICo through a diencephalic periventricular and central mesencephalic grey system and has proposed that the intercollicular area modulates the efferent flow from tectal systems for positive targetting, and/or enhances the tectal processes themselves. Thus, much of the chick lesion data can be understood in terms of tectal and brainstem systems for head and body orientation for visual fixation which are facilitated through an archistriatal-intercollicular pathway. The non-sexual behavioural effects of testosterone can then be seen as the results of selective facilitation of these tectal orientation systems, and the uptake of this hormone by the ICo could account for these effects.

The occurrence of a similar uptake of testosterone by the IO is interesting because this nucleus projects solely to the retina, and according to the electrophysiological studies of Miles<sup>13</sup>, activity in this nucleus effectively enhances retinal responses to both large and small stimuli by removing inhibitory surrounds and facilitating the excitatory centres of retinal ganglion cell response fields. Miles has suggested that the centrifugal system provides a rapid local transient dark-adaptation system for improved detection in shadowed areas during visual search. This is consistent with the findings of Rogers and Miles<sup>14</sup>, that lesions of the IO impaired the discrimination of food from sand grains in the darker areas of a variegated brightness field. These birds also pecked at food and non-food objects in an apparently indiscriminate and exploratory fashion with closed bill. They were also poorer at responding to a novel object introduced into the posterior visual field while feeding. These observations suggest an impairment of object recognition or target distinctiveness, permitting less selectivity within the attentional system and resulting in more general behavioural effects than would the loss of a local dark-adaptation system. We suggest, therefore, that androgen uptake in the IO increases target distinctiveness and so contributes to the observed enhanced visual orienting and attentional behaviour of the hormone-injected chick. The interest in both these midbrain nuclei being androgen sensitive is that they are parts of neural systems that could operate in orientation behaviour to specific focal visual stimulation

and so provide a neural basis for the perceptual and "non-sexual" behavioural effects of testosterone.

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## Interspecific variation in products of animal mitochondrial protein synthesis

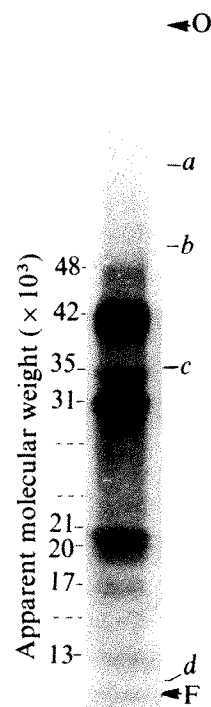
THE DNA of mammalian mitochondria (mtDNA) exists as a single class of circular molecules about 5  $\mu$ m long<sup>1,2</sup>, and codes for two ribosomal RNA species and several transfer (4S) RNA components<sup>3,4</sup>. The function of the remaining 80% of the genome is unknown, although it has been suggested that the residual coding capacity provides messenger RNA (mRNA) which is translated exclusively by mitochondrial ribosomes producing about ten protein components which are associated with the inner mitochondrial membrane. Not all protein synthesis in mitochondria, however, may be directed by mtDNA—some could represent translation products of nuclear-coded messages imported into the organelles<sup>5,6</sup>. We report here on differences between electrophoretic profiles of proteins synthesised in mitochondria of a wider variety of animal cell lines. Evidence is also presented suggesting that mtDNA has a role in determining the pattern of proteins observed.

It is widely assumed that the proteins synthesised in cells treated with inhibitors of cytoplasmic protein synthesis are of mitochondrial origin. We have shown previously that about ten major proteins are selectively labelled by culturing cells in the presence of <sup>35</sup>S-methionine and the inhibitor emetine<sup>7,8</sup>. The labelled proteins copurified with mitochondria on cell fractionation and their synthesis was blocked by chloramphenicol, a known inhibitor of mitochondrial protein synthesis. Our preliminary investigations demonstrated that the patterns for human and mouse mitochondrially-synthesised proteins, as resolved by electrophoresis in sodium dodecyl sulphate (SDS)-polyacrylamide gels, were different<sup>7</sup>.

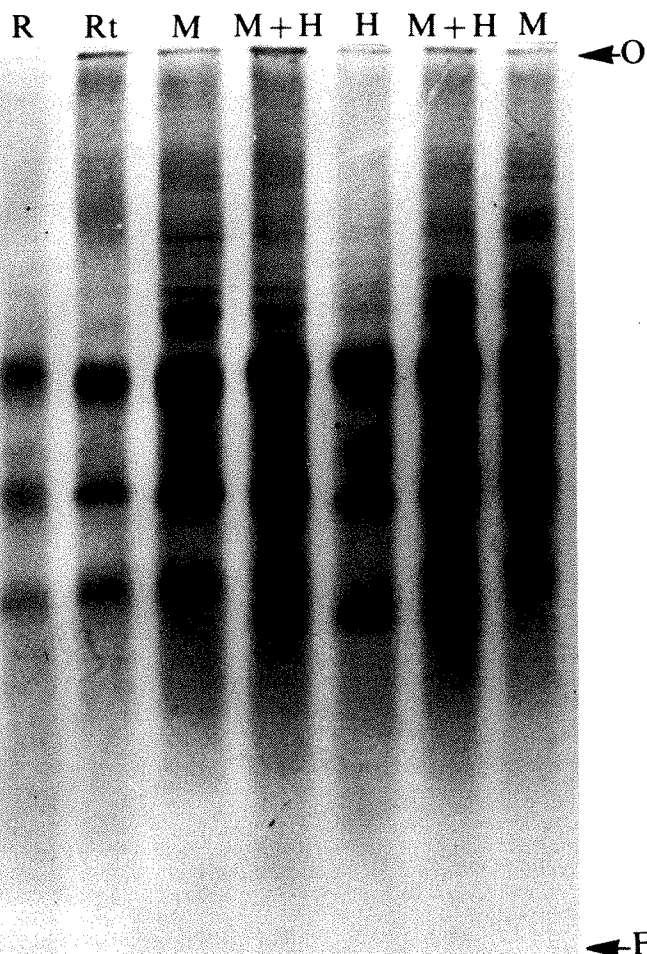
A typical profile of the proteins detected by the technique of selective labelling followed by electrophoresis and autoradiography is illustrated in Fig. 1. This pattern obtained with human cells shows about eight predominant bands which were labelled in the presence of the cytoplasmic protein synthesis inhibitor emetine. The approxi-

mate molecular weights were determined by calibrating the gel with marker proteins of known size.

Using cells from various species, we have observed some differences in the profiles for animals more closely related than humans and mice. Thus, among the rodents (Fig. 2), profiles from rat and mouse cell lines were similar but showed small, reproducible differences from those of both hamster and rabbit. With other animal cells, there were variations in the electrophoretic mobilities of most labelled bands (Fig. 3). The total number of bands (7-11) and their molecular weight range (10,000-50,000), however, were fairly constant. Some of the components detected were common to profiles of most species examined—for example, the band of apparent molecular weight 45,000—whereas others varied considerably so that homologies were difficult to establish. The patterns for man, chimpanzee and the African green monkey showed an overall similarity, but differed somewhat from those of marmoset (a New World monkey). In all species studied, no more than about 3  $\mu$ m DNA would be required to code for these mitochondrially synthesised proteins (based on apparent molecular weight determinations). This is within the coding capacity of animal mtDNA. Bands of molecular weight above 50,000 were generally not sensitive to chloramphenicol. Furthermore, experiments with human cells demonstrated that



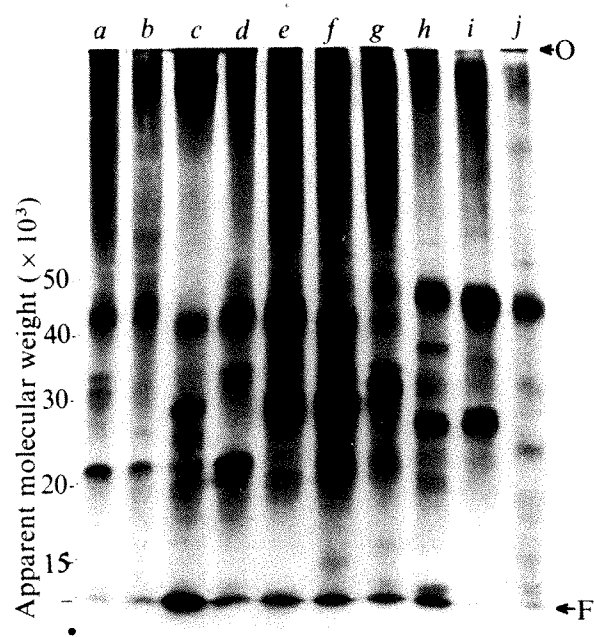
**Fig. 1** Electrophoretic profile of proteins synthesised *in vivo* by mitochondria of human cells. HeLa cells, labelled with <sup>35</sup>S-methionine for 4 h in presence of 50  $\mu$ g ml<sup>-1</sup> emetine, were solubilised in buffer containing SDS and 2-mercaptoethanol. Samples were electrophoresed on a 12% (w/v) polyacrylamide slab gel and labelled polypeptides detected subsequently by autoradiography. Details of procedures for incorporation, electrophoresis and autoradiography have been described previously<sup>7</sup>. Marker proteins (*a*, bovine serum albumin; *b*, bovine glutamate dehydrogenase; *c*, rabbit lactate dehydrogenase; *d*, equine cytochrome *c*) were run on the same slab gel to provide a calibration for molecular weight determination. Labelled material of molecular weight about 42,000 consists of two closely spaced bands. Additional components which are sometimes detected in the system are marked by dashed lines. Addition of phenylmethylsulphonylfluoride (1 mM) before solubilisation was found not to affect band pattern observed. Errors in molecular weight assignment could result from differences in hydrophobicity between marker and mitochondrial proteins. O, Origin; F, front.



**Fig. 2** Electrophoretic profile of mitochondrially-made proteins from various rodent cell lines. R, Rabbit; Rt, rat; M, mouse; H, Chinese hamster. Cells were labelled with  $^{35}\text{S}$ -methionine for 4 h in presence of  $50 \mu\text{g ml}^{-1}$  emetine, before solubilisation, electrophoresis and autoradiography. O, Origin; F, front.

they could be eliminated by purification of mitochondria before electrophoresis. These bands of higher molecular weight may represent the residual products of cytoplasmic protein synthesis.

We have also examined the spectrum of proteins synthesised by mitochondria of human-mouse somatic cell hybrids. Profiles obtained with 17 independently-derived hybrid cell lines were similar to those of mouse parental cell lines. No human components, which could be distinguished from the mitochondrially-synthesised proteins of the mouse, were detected. Similar observations on four such hybrid cell lines have been reported previously<sup>7</sup>. Karyotypic analysis indicated that every human chromosome (with the possible exception of the Y) was represented in at least one of the hybrids examined (ref. 9 and E. Solomon *et al.*, unpublished). Since human mtDNA seems to be eliminated from man-mouse hybrids in which the mouse parent is an established cell line<sup>10,11</sup>, the detection of only mouse mitochondrially-synthesised proteins is consistent with the suggestion that the genes for at least some of the human proteins can be assigned to mtDNA. The absence of human mitochondrial proteins would thus reflect the loss of human mtDNA. Other interpretations based on the possible existence of dominant modification



**Fig. 3** An electrophoretic comparison of proteins synthesised by mitochondria of various animal cell lines. a, Man; b, chimpanzee; c, marmoset; d, African green monkey; e, cat; f, mouse; g, marsupial mouse (*Sminthopsis*); h, chicken; i, *Xenopus laevis*; j, *Drosophila melanogaster*. Cell lines were labelled for 4 h in presence of  $100 \mu\text{g ml}^{-1}$  emetine. Incorporations were at  $37^\circ\text{C}$  except for *Xenopus* and *Drosophila* cells ( $25^\circ\text{C}$ ). Incorporation medium for *Drosophila* embryonic cells was D22 medium<sup>17</sup>, in which yeastolate and lactalbumin hydrolysate were replaced by  $67 \mu\text{M}$  L-amino acids minus methionine. Further supplements were 0.5% (v/v) foetal calf serum and 9.5% (v/v) dialysed serum<sup>7</sup>. Details of cell lines used may be obtained from the authors on request. O, Origin; F, front.

mechanisms controlled by genes on mouse chromosomes, or on the selective repression of human nuclear genes for these proteins, can be envisaged, but require further, as yet untested, assumptions.

The observed variation in the profiles of mitochondrially-synthesised proteins in the several species examined therefore may reflect divergence in the corresponding structural genes in the mitochondrial genome. In this context, animal mitochondria have been shown to synthesise about eight species of poly(A)-containing RNA, which seem to represent messages transcribed from mtDNA. Not only does this number of messages correspond roughly to the number of proteins in this study, but there is interspecies variation in the sizes of these RNA components<sup>12,13</sup>. Furthermore, extensive divergence in the nucleotide sequences of mtDNA from various animal species has been reported<sup>14-16</sup>.

Our electrophoretic analysis indicates substantial variation in the size and/or composition of mitochondrially-synthesised proteins of different animal cell lines and a possible role of postsynthetic modification (for example, glycosylation, cleavage or aggregation) should be considered. Nevertheless, the reproducibility and stability of the profiles, together with information from the genetic analysis with human-mouse hybrid cells, suggests a lack of conservation of the proteins synthesised in animal mitochondria. Caution should therefore be exercised in extrapolations concerning their nature and function based on information from the lower eukaryotes—for example, yeast.

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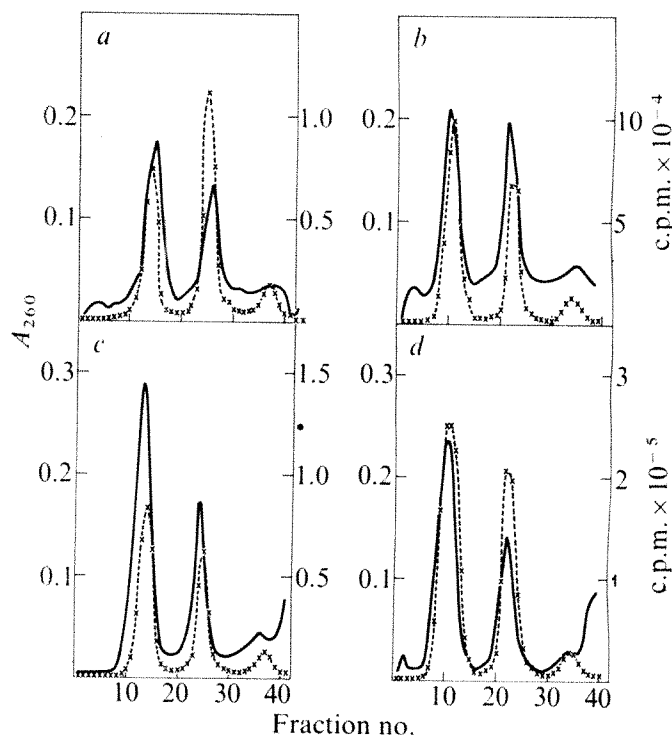
## Ribosome metabolism in temperature-sensitive mutant of BHK cells

CELLULAR content of ribosomes is coordinately regulated with cell growth<sup>1</sup>, either at the level of ribosome processing or ribosomal degradation. The two ribosomal subunits in growing mammalian cells are synthesised in equimolar amounts as cleavage products of a common precursor<sup>2</sup>; and it also seems that all the components of ribosomes are degraded as a unit<sup>3</sup>. The regulatory mechanism of ribosome metabolism in mammalian cells, however, remains unclear.

We have studied ribosome metabolism in the temperature-sensitive mutant, *ts422E*, derived from BHK cells, that are defective in the processing of large ribosomal subunits (28S rRNA) at the non-permissive temperature (39 °C)<sup>4,5</sup>. Synthesis of the small ribosomal subunits (18S rRNA), on the other hand, is uninhibited. The resulting transient imbalance in the ribosomal subunits at 39 °C seems to be corrected by a higher rate of degradation of the 18S rRNA in the cytoplasm. As the rate of accumulation of poly(A)-containing RNA in the polyribosomes, and the rate of protein synthesis in *ts422E* remain unchanged at 39 °C, it seems that the instability of a fraction of 18S rRNA in the mutant cells arises from their lack of participation in protein synthesis.

When shifted to the non-permissive temperature, the *ts422E* cells show a transient increase in cell number followed by an actual decline in viable cells compared with those cultures remaining at the permissive temperature (Fig. 2a, inset). Although the total ribosome content in *ts422E* cells show equimolar amounts of 28S and 18S rRNA after 25 h at 39 °C, the amounts of newly synthesised 28S rRNA at the non-permissive temperature is markedly reduced compared with cells at the permissive temperature (Fig. 1a and c). When the radioactivity in rRNA synthesised at 39 °C is chased with excess unlabelled uridine and cytidine for 42.5 h at 39 °C, however, the specific activities of 28S and 18S rRNA approach that of *ts422E* cells maintained at the permissive temperature (Fig. 1b and d). Therefore, the 18S rRNA accumulated in *ts422E* soon after shift up to 39 °C must be balanced by its faster rate of degradation. This possibility was tested directly by comparing the rate of decay of 18S and 28S rRNA in *ts422E* cells at the permissive and non-permissive temperatures.

The radioactivity in 18S rRNA from *ts422E* cells labelled

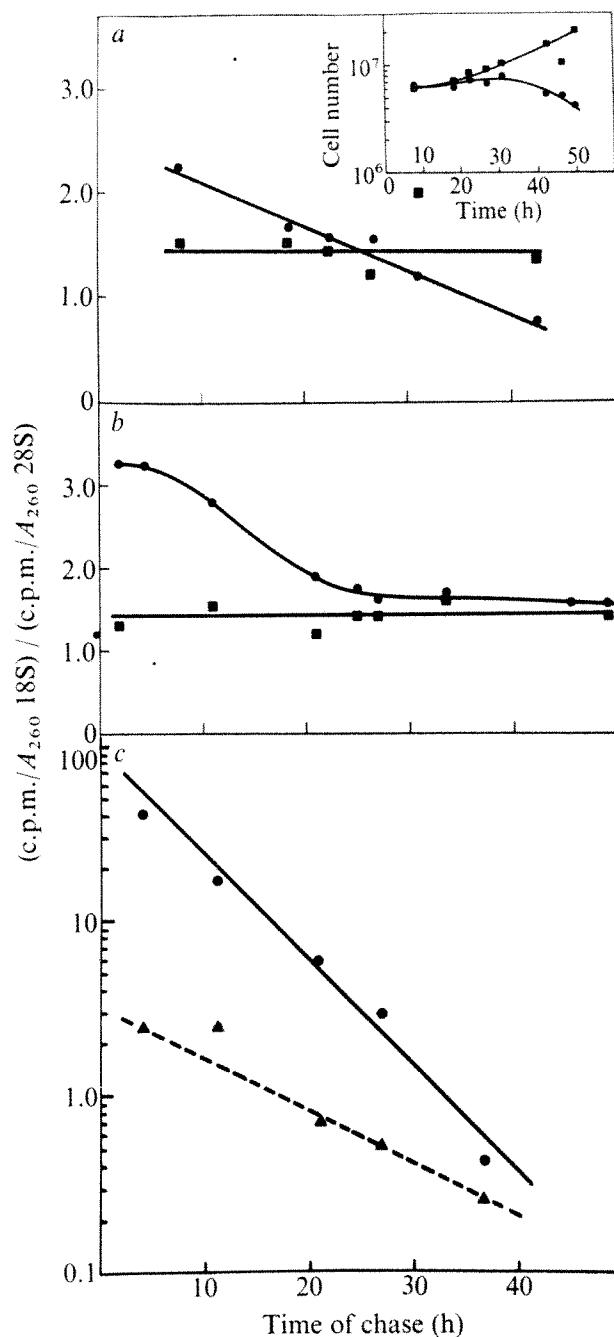


**Fig. 1** RNA synthesis in *ts422E* at 39 °C. Cultures of *ts422E* cells were maintained at 33 °C in Dulbecco-Vogt modified Eagle's Medium (DME) supplemented with 10% calf serum. Eight 100-mm culture dishes were shifted to 39 °C and labelled between 15 and 17 h with 10  $\mu$ Ci ml<sup>-1</sup> <sup>3</sup>H-uridine (specific activity 25 Ci mmol<sup>-1</sup>). After <sup>3</sup>H-uridine incubation, labelled RNA was chased by substituting the medium with fresh DME supplemented with 5 mM uridine plus 2.5 mM cytidine at 39 °C for 8 h (a) and 42.5 h (b). Control cultures maintained at the permissive temperature were pulse-labelled and chased as above (c and d). The chase was terminated by washing cells with chilled (2–4 °C) balanced salt solution (Earle's). Cells were detached by trypsin treatment, washed twice with cold Earle's solution and disrupted by Dounce homogenisation in RSB buffer (0.01 M Tris-HCl, pH 7.6, 0.01 M NaCl, 1.5 mM MgCl<sub>2</sub>). Nuclei and large cell particulates were eliminated by successive centrifugations at 1,000g for 3 min and 12,000g for 10 min, and the ribosomes were precipitated from the postmitochondrial supernatant with the addition of 70 mM MgCl<sub>2</sub> for 45 min at 4 °C (ref. 10). Ribosomes were pelleted by centrifugation at 12,000g for 10 min and the pellets resuspended in 5 ml NETS buffer (0.01 M Tris-HCl, pH 9.0, 0.01 M EDTA, 0.1 M NaCl, 0.2% SDS) and the RNA extracted with equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) until no visible interphase was present. RNA was precipitated from the aqueous phase with two volumes of ethanol, collected by centrifugation and resolved by centrifugation in 15–30% sucrose gradients in NETS buffer. Samples were centrifuged in a Spinco SW27 rotor at 23 °C and 23,000 r.p.m. for 16 h. Gradients were eluted through a recording spectrophotometer, and samples were prepared for the determination of acid-insoluble radioactivity and counted in a liquid scintillation counter as described<sup>10</sup>. a, 8-h chase at 39 °C; b, 42.5-h chase at 39 °C; c, 8-h chase at 33 °C; d, 42.5-h chase at 33 °C. —,  $A_{260}$ ; x, c.p.m.

with <sup>3</sup>H-uridine between 15 and 17 h at 39 °C, decays at a faster rate than the radioactivity in 28S rRNA when chased in a growth medium containing excess unlabelled uridine and cytidine at 39 °C. During a similar chase period of 40–50 h there is little difference in the relative rates of decay of 18S and 28S rRNA of the mutant cells that remained at the permissive temperature (Fig. 2a). The enhanced decay of 18S rRNA in the temperature-sensitive mutants seems to be a transient adjustment to the non-permissive condition. As shown in Fig. 2b, the faster decay rate of <sup>3</sup>H-methylmethionine pulse-labelled 18S rRNA at 39 °C, on being chased with excess unlabelled methionine at 33 °C, approaches the rate of rRNA degradation in the cells that were labelled and chased at the permissive temperature. This apparent recovery from the faster rate of

**Fig. 2** Rates of decay of 18S and 28S rRNA. *a*, *ts422E* cells maintained at 33 °C were shifted to 39 °C and pulse labelled with  $^3\text{H}$ -uridine between 15 and 17 h as described in Fig. 1. Control cultures maintained at 33 °C were similarly pulse labelled for 2 h. After pulse label, the growth medium was replaced with DME containing excess unlabelled uridine and cytidine, and cells collected at indicated times after the chase. rRNA from cells after various chase periods was fractionated, and the 18S and 28S rRNA resolved on NETS-sucrose gradients as in Fig. 1. Specific activities were determined by integrating the areas under the  $A_{260}$  profiles of 18S and 28S rRNA, and adding the radioactivities of the two rRNA species. The change in the relative specific activities of rRNAs for cultures kept at 33 °C and those at 39 °C are shown: ■, 33 °C; ●, 39 °C. Inset shows changes in cell number of the cultures maintained at 33 °C and 39 °C. *b*, Cultures of *ts422E* cells were shifted to 39 °C in normal growth medium and pulse labelled with 25  $\mu\text{Ci ml}^{-1}$   $^3\text{H}$ -methylmethionine (specific activity 300 mCi mmol $^{-1}$ ) for 30 min (between 16.5 and 17 h at 39 °C) in DME lacking methionine but containing 10% calf serum. The medium was then replaced with a chase medium (DME containing 8 mM methionine plus 10% calf serum), and labelled cells restored to the permissive temperature and allowed to grow at 33 °C. Control samples *ts422E* cells maintained at 33 °C were similarly pulse labelled with  $^3\text{H}$ -methylmethionine and also chased at 33 °C. At indicated times after the beginning of the chase period, samples from cells pulse labelled at 39 and 33 °C were removed. Cells collected and rRNA purified and resolved on sucrose gradients as in Fig. 1. Relative rates of decay in the specific activities of rRNAs were estimated by integrating the areas under the  $A_{260}$  profile and the radioactivity under the 28S and 18S rRNA, as in *a*. *c*, Ten 100-mm culture dishes of *ts422E* cells were labelled with  $^{14}\text{C}$ -methylmethionine (5  $\mu\text{Ci ml}^{-1}$ , specific activity 50 mCi mmol $^{-1}$ ) for 24 h at 33 °C in DME plus 10% calf serum. After steady-state labelling with  $^{14}\text{C}$ -methionine, the medium was replaced with fresh DME and the cells grown for the first 16 h at 33 °C and then shifted to the non-permissive temperature (39 °C) for another 16 h. Cells were then pulse labelled with 20  $\mu\text{Ci ml}^{-1}$   $^3\text{H}$ -methylmethionine (specific activity 230 mCi mmol $^{-1}$ ) for 1.5 h at 39 °C and chased with fresh DME supplemented with 10% calf serum and 8 mM L-methionine. Samples were taken at indicated times after the beginning of chase at 39 °C; cells were collected and rRNAs prepared and resolved on sucrose gradients as above. The radioactivity in each rRNA species was normalised to the  $A_{260}$  of total cell DNA prepared according to Scott *et al.*<sup>11</sup>. The illustration compares the relative rate of decay of specific activities in rRNAs labelled before ( $^{14}\text{C}$ ) and after ( $^3\text{H}$ ) the shift up to non-permissive temperature.

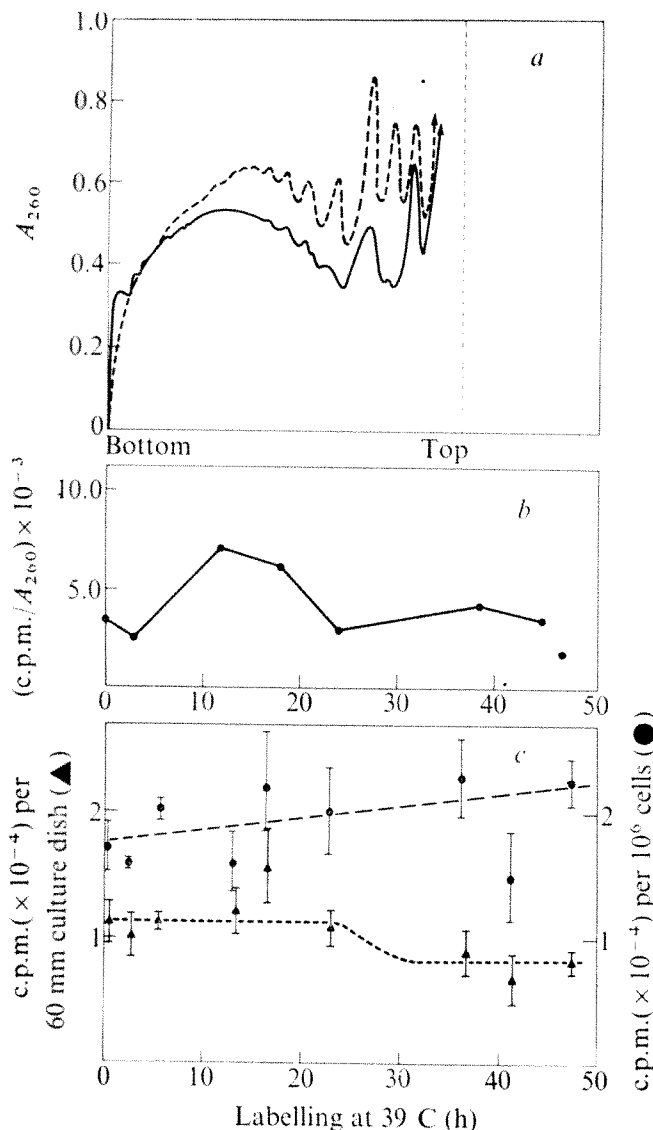
decay of rRNA at 39 °C is complete within about 24 h of restoration of the permissive growth conditions (Fig. 2b). Since the experiments so far have shown an accelerated rate of decay of 18S rRNA after a shift up to 39 °C, it is of interest to examine whether the 18S rRNA synthesised at the non-permissive temperature is preferentially degraded. Although both the existing 18S rRNA ( $^{14}\text{C}$ -labelled) synthesised at the permissive temperature and that synthesised after the shift up to 39 °C ( $^3\text{H}$ -labelled) are degraded at a faster rate than the corresponding 28S rRNA, the rate of decay of 18S rRNA made after the shift to 39 °C was



**Table 1** Appearance of 18S rRNA in cytoplasm of BHK and 422E cells

Time	New		Polyribosomal Old		Ratio		New		Native subunits Old		Ratio	
	$^3\text{H}$ (c.p.m./10 <sup>7</sup> cells)	422E	BHK	422E	BHK	422E	BHK	422E	BHK	422E	BHK	422E
30 min	—	72	—	213	—	0.33	437	508	770	929	0.56	0.83
60 min	395	192	243	220	1.62	0.87	2,052	1,871	886	506	2.31	3.70
90 min	1,085	3,060	381	1,257	2.85	2.43	8,452	2,866	1,256	493	6.73	5.81
24 h	11,189	4,654	2,105	1,370	5.32	3.39	5,080	5,622	763	1,023	6.65	5.50

Proportions of old and new 18S rRNA within polysomal and native subunit fraction of BHK and 422E cells determined by incubating cultures of both types of cells at permissive temperature with 2.5  $\mu\text{Ci ml}^{-1}$   $^{14}\text{C}$ -methylmethionine (specific activity 58 mCi mmol $^{-1}$ ) in complete medium for 24 h. Growth medium then replaced twice within 8 h with fresh DME at 33 °C and cultures transferred to 39 °C. After 17 h at non-permissive temperature, cells pulse labelled with 25  $\mu\text{Ci ml}^{-1}$   $^3\text{H}$ -methylmethionine in DME lacking methionine and samples removed at 30, 60 and 90 min thereafter. Remaining fraction of each of labelled BHK and *ts422E* cultures washed and grown for 24 h in chase medium containing excess (40 times normal concentration in DME) unlabelled methionine. Cells collected, homogenised and postmitochondrial supernatant resolved through 8 ml 5–20% (w/w) sucrose gradient in RSB, layered over a 2-ml cushion 60% sucrose in RSB, and centrifuged in a Spinco SW41 rotor at 36,000 r.p.m. for 3.5 h at 4 °C. Polysomal and native 40S subunit regions collected and RNA phenol extracted as before, purified by oligo-d(T)-cellulose chromatography, and poly(A)-lacking RNA further resolved on 15–30% sucrose-NETS gradients as in Fig. 1. Radioactivity in old ( $^{14}\text{C}$ ) and new ( $^3\text{H}$ ) 18S rRNA from each sample corrected for background and spill, normalised to number of cells in each fraction.



**Fig. 3** Polyribosomal RNA and protein synthesis in *ts422E* at 39 °C. *a*, Cultures *ts422E* cells maintained at 33 °C shifted to 39 °C for 3 h (broken line) and 24 h (solid line). Cells were collected, washed and disrupted by Dounce homogenisation in RSB buffer as described<sup>10</sup>. Postmitochondrial supernatant was prepared by centrifugation at 12,000 *g* for 10 min, and polyribosomes from the supernatant resolved by centrifugation in 15–30% (w/w) sucrose gradient in RSB. Centrifugation was done in a Spinco SW27 rotor at 26,000 r.p.m. for 2.5 h and 4 °C, and the sample eluted through a continuously recording spectrophotometer.  $A_{260}$ ; —, 3 h at 39 °C; - - - - -, 24 h at 39 °C. *b*, Polysomal poly(A)-containing RNA was estimated from cultures labelled for 2 h with <sup>3</sup>H-adenosine (50  $\mu$ Ci ml<sup>-1</sup>, specific activity 40.8 Ci mmol<sup>-1</sup>) before isolating polyribosomes as in *a*. Pooled polyribosomes were extracted twice with phenol-chloroform-isoamyl alcohol (50:50:1) followed by extraction with chloroform-isoamyl alcohol (96:4) and precipitated with two volumes of 95% ethanol.  $A_{260}$  was determined on sample aliquots. Incorporation of adenosine into polysomal poly(A) was measured by incubating samples with T<sub>1</sub> RNase (20 U ml<sup>-1</sup>) and RNase A (20  $\mu$ g ml<sup>-1</sup>) at 37 °C for 20 min in 0.5 M NaCl and 0.01 M Tris-HCl buffer. The mixture was run through an oligo(dT)-cellulose column and washed extensively with the same buffer. The amount bound was determined by eluting poly(A) with 0.1 M Tris, pH 7.4, buffer and determining the c.p.m. in the eluate. ●, c.p.m. in polysomal poly(A)/ $A_{260}$  of polysomal RNA. *c*, Rate of protein synthesis was determined in 60-mm cultures of *ts422E* cells shifted to 39 °C and pulse labelled with 2  $\mu$ Ci ml<sup>-1</sup> <sup>3</sup>H-L-amino acid mixture (NET-250) for 1 h. Each point represents an average of three determinations (vertical bar represents the range of counts in each of the three 60-mm cultures). The first time point was taken soon after the shift from 33 to 39 °C and the subsequent ones at indicated time intervals after transfer to 39 °C. Incorporation was terminated by the addition of 5 ml cold (2–4 °C) balanced salt solution, and cells collected and washed as before. Cells were then lysed and prepared for the determination of acid-insoluble radioactivity as described<sup>10</sup>.

faster than that of the pre-existing 18S rRNA (Fig. 2c).

As shown in Fig. 3a, there is an increase in the amount of free 40S subunits, which presumably represents the excess 18S rRNA not yet utilised in protein synthesis. Since there is no shift towards slower-sedimenting polyribosomes at the non-permissive temperature (Fig. 3a), it is likely that the increased quantity of free 40S subunits in *ts422E* after 24 h at 39 °C represents an inhibition of translational initiation.

The rate of accumulation of poly(A)-containing (poly(A)<sup>+</sup>) pulse-labelled polyribosomal RNA is *ts422E* (Fig. 3b) is not significantly altered after 40 h at the non-permissive temperature. The initial increase in the accumulation of poly(A)<sup>+</sup> polyribosomal RNA may represent an enhanced rate of incorporation in acid-precipitable RNA at 39 °C compared with 33 °C since there is no change in the acid-soluble pool at the two temperatures (A.K., E.B. and W.T.M., unpublished). Thus it seems that there is little change in the rate of messenger RNA (mRNA) processing in the temperature-sensitive mutants at the non-permissive temperature.

The rate of protein synthesis in *ts422E* cells is slightly increased when shifted from 33 to 39 °C (Fig. 3c), an effect similar to that of increased incorporation of acid-precipitable RNA at 39 °C. On a per-culture basis, however, there is a slight decline in the rate of protein synthesis after about 25 h at 39 °C, largely because of the loss in the number of viable mutant cells on prolonged incubation at 39 °C (Figs 3c and 2a, inset).

The fact that pre-existing small ribosomal subunits are degraded at a slower rate at 39 °C than those synthesised after shift up to the non-permissive temperature (Fig. 2c) may suggest that the pre-existing ribosomes, having been used in protein synthesis, are more stable than the nascent ribosomes.

An estimate of the amounts of existing 18S rRNA and the new 18S rRNA synthesised at 39 °C in the polyribosomal and native subunit fractions (Table 1) further suggests that more existing 18S rRNA enters the polyribosomes in *ts422E* cells at the non-permissive temperature than in the wild-type cells within 90 min of equilibration.

During the transition from resting to growing fibroblasts there is a marked increase in the stability of rRNA<sup>6,7</sup>. One of the early events in the re-initiation of cell growth seems to be an increased use of ribosomes<sup>8,9</sup>, indicated by the enhanced rate of protein synthesis. Since the two ribosomal subunits are functionally coupled in mRNA translation, it seems that the stability and thus the control of degradation of ribosomes in mammalian cells depends on their use in protein synthesis.

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## Magnitude and significance of NAD turnover in human cell line D98/AH2

In the oxidation-reduction metabolism of a cell the pyridine nucleotide NAD is used catalytically; any NAD that is reduced is reoxidised. But NAD is consumed in certain metabolic reactions in which it serves as a substrate. In eukaryotic cells, the most intriguing of these reactions is the cleavage of NAD to form nicotinamide and a unique polymer, poly adenosine diphosphoribose (poly ADPR) (Fig. 1); the reaction is catalysed by the enzyme poly ADPR synthetase<sup>1-4</sup>. Other well studied reactions involving the destruction of NAD include the cleavage of NAD to form AMP and NMN by bacterial DNA ligases<sup>5,6</sup> and the breakdown of NAD (with the concomitant inactivation of protein synthesis) by diphtheria toxin<sup>7,8</sup>.

What is the magnitude *in vivo* of the reactions which consume NAD? We present here data for D98/AH2, a human cell line derived from HeLa<sup>9</sup> which answers this question and which makes possible calculation of the fraction of NAD biosynthesis that compensates for breakdown and the fraction that expands the NAD pool during growth.

D98/AH2 cells were grown in medium containing <sup>14</sup>C-adenine and <sup>3</sup>H-nicotinic acid, both precursors of NAD, for 6 h and then moved to unlabelled medium. At various times after transfer, intracellular nucleotides were extracted with acid and analysed by paper chromatography. The data obtained from one such experiment are shown in Table 1. These data permit a precise calculation of the breakdown rate of NAD *in vivo* (Table 2). Several experiments of this general type have been performed; all yield values for the *in vivo* half life of NAD of about  $1.0 \pm 0.3$  h for D98/AH2 cells<sup>10</sup>. This value is equivalent to a breakdown rate of  $96,000 \pm 30,000$  per s per cell. Because the generation time of D98/AH2 is 24 h and the NAD pool size is  $5 \times 10^8$  molecules per cell (ref. 11), a net synthesis

Table 1 Relative amount of <sup>14</sup>C-adenine in ATP and NAD pools as a function of time

Time (h)	<sup>14</sup> C-adenine in: NAD pool ( $D_t/D_0$ ) (expressed as a ratio to the radioactivity at $t=0$ )	ATP pool ( $A/A^0$ )
0	1.0	1.0
1	0.87	0.86
3	0.70	0.47
8	0.39	0.24

Four equivalent D98/AH2 cultures ( $\sim 2 \times 10^6$  cells) were grown in 5 ml Falcon flasks for 8 h in F12 medium which contained  $0.2 \mu\text{g ml}^{-1}$  nicotinic acid ( $0.1 \mu\text{g ml}^{-1}$ ,  $5 \times 10^8$  c.p.m.  $\mu\text{mol}^{-1}$ ) and <sup>14</sup>C-adenine ( $0.2 \mu\text{g ml}^{-1}$ ,  $6.6 \times 10^7$  c.p.m.  $\mu\text{mol}^{-1}$ ); cells were then washed and allowed to grow in unlabelled F12 medium which contained nicotinic acid  $0.1 \mu\text{g ml}^{-1}$  and adenosine ( $20 \mu\text{g ml}^{-1}$ ) for the time indicated. Nucleotides were acid-extracted from the cells and analysed by chromatography on DEAE paper as described previously<sup>11</sup>. The total <sup>14</sup>C in the ATP peak and <sup>3</sup>H and <sup>14</sup>C in the NAD peak was calculated for each chromatogram. The <sup>14</sup>C-adenine in the ATP and NAD pools could then be determined, using the <sup>3</sup>H in NAD to normalise to time ( $t$ ) zero. The amount of radioactivity in each pool for the culture collected at  $t=0$  is: <sup>14</sup>C-adenine in ATP, 159,000 c.p.m.; <sup>14</sup>C-adenine in NAD, 21,000 c.p.m.; <sup>3</sup>H-nicotinamide in NAD, 165,000 c.p.m.

rate of only 4,000 molecules per s per cell is needed to maintain the pool size of NAD during growth. Thus, the true rate of NAD biosynthesis is approximately  $10^5$  molecules per s per cell and about 95% of this replaces the NAD that is catabolised, and only 5% maintains the NAD pool size during growth.

We have already studied NAD turnover in enucleated D98/AH2 cells<sup>12</sup>. Cells were grown in <sup>3</sup>H-nicotinic acid, enucleated by cytochalasin treatment and centrifugation<sup>13</sup>, and then incubated in unlabelled medium. The rate of loss of <sup>3</sup>H-NAD was measured by an autoradiographic method developed for pyridine nucleotides<sup>14</sup>. The observed half life of the labelled NAD in enucleated cells was 10 h (ref. 12). Because enucleated cells cannot synthesise NAD<sup>12</sup>, the observed 10 h half life of the label is a direct measure of

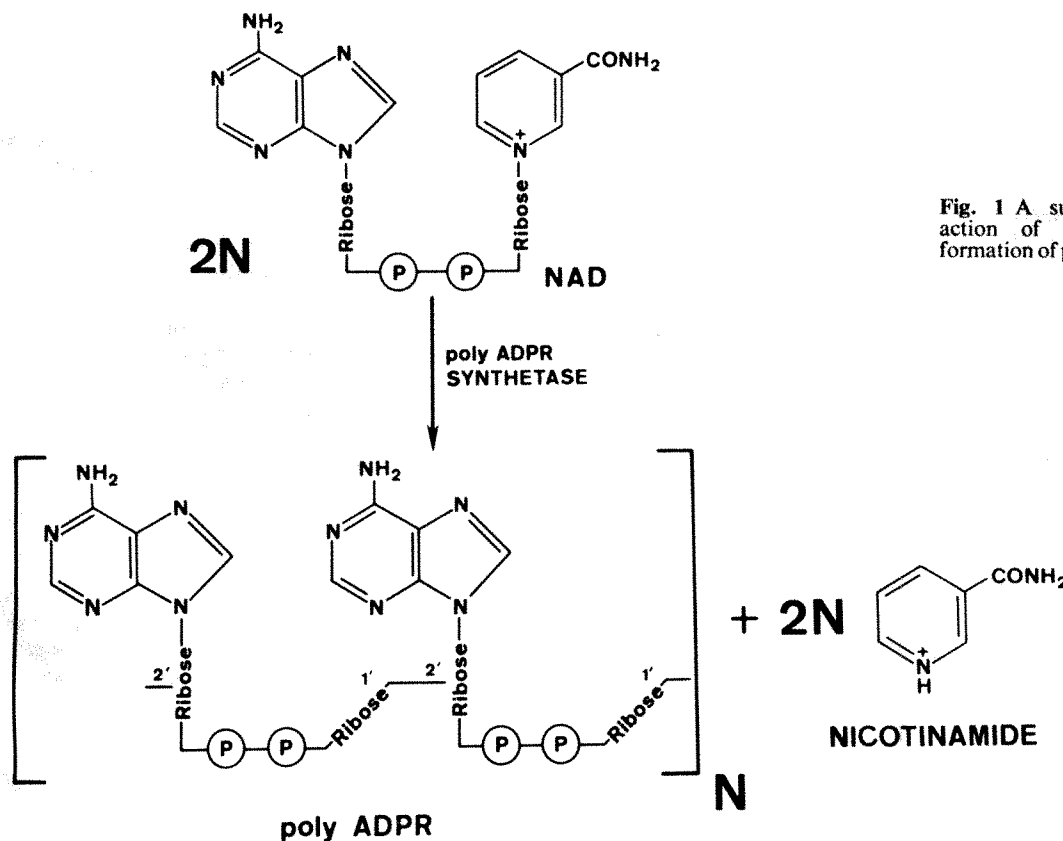


Fig. 1 A substrate reaction of NAD: the formation of poly ADPR.



**Table 2** Constants calculated from the experimental data in Table 1

	Adenine in NAD ( $D$ )	Adenine in ATP ( $A$ )
Apparent decay constant, $K$	$3.3 \times 10^{-5} \text{ s}^{-1}$	$5.2 \times 10^{-5} \text{ s}^{-1}$
Apparent half life	5.8 h	3.7 h

True rate of breakdown of NAD,  $R_B$ : 124,000 molecules per s per cell  
 True half life for adenine in NAD,  $\tau$ : 0.78 h  
 $R_B$  was estimated by the equation:

$$\ln D_t/D_0 = -\frac{R_B t}{n_D} + \frac{(R_u + R_B)\{1 - \exp[-(K_A - K_D)t]\}}{(K_A - K_D)}$$

where  $D_t$ ,  $D_0$  are the radioactivity of adenine in the NAD pool at times  $t$  and 0 respectively, and  $K_A$  and  $K_D$  are the decay constants above,  $n_D$  is the pool size of NAD (no. of molecules per cell),  $R_u$  the number of NAD molecules synthesised to increase NAD pool size during growth. The quantity  $(R_u + R_B)$  therefore represents the total rate of NAD synthesis. A derivation of this equation with a complete kinetic analysis of this and similar data is represented elsewhere<sup>10</sup>.

$$\tau = \frac{0.693 n_D}{R_B}$$

the rate of breakdown of NAD. As described above, NAD has a 1 h half life in nucleated cells. Together, these observations strongly indicate that NAD breakdown occurs primarily in the nucleus, although other more complex possibilities cannot be rigorously excluded.

The picture begins to emerge of the nucleus as an organelle in which NAD is actively synthesised and broken down. It has been known for many years that NAD pyrophosphorylase, one of the key NAD biosynthetic enzymes, is located within the nucleus<sup>15</sup>. Indeed, this enzyme is used as the characteristic marker enzyme of chromatin<sup>19</sup>. The data reported here, which indicate that most NAD biosynthesis compensates for NAD breakdown in the nucleus provide a rationale for the well established location of this NAD biosynthetic enzyme.

In isolated nuclei, poly ADPR synthetase is the major enzyme causing NAD breakdown<sup>3,4</sup>. Although direct and conclusive evidence is not available, we suggest that this also holds true in the intact cell. Several features of the reaction catalysed by the synthetase suggest that the enzyme may be involved in nuclear function. The enzyme requires DNA and histones for activity<sup>17</sup>. The product of the reaction, poly ADPR, which incidentally, is both polynucleotide and polysaccharide, has been reported to be covalently bound to histone F1 (ref. 18). The synthetase has been found in all types of eukaryotic cells, from the slime mould *Physarum*<sup>19</sup> to human tissue culture cells; this is consistent with a vital (and therefore evolutionarily conserved) role for the enzyme in the eukaryotic nucleus.

Our measurements indicate a surprisingly high rate of NAD breakdown; during one cell cycle, for example, there is twice as much adenine leaving NAD as is being incorporated into DNA. If poly ADPR synthesis were indeed responsible for a substantial fraction of NAD breakdown, then the amount of poly ADPR synthesised in one cell cycle would be of the same order of magnitude as the amount of DNA synthesised. A high steady-state level of poly ADPR, however, would not necessarily accumulate if breakdown were rapid. Indeed, two enzymes able to degrade poly ADPR have been isolated from mammalian nuclei<sup>20,21</sup>.

The existence of a cellular NAD turnover cycle was first proposed by Gholson in 1966 (ref. 22). He argued that such a cycle "... had an important but as yet unknown function ... in cellular metabolism". This statement still applies; it seems likely that in eukaryotic cells, NAD has some other major function in addition to the classical cytoplasmic role in oxidation and reduction (which utilises

the nicotinamide moiety). Our results indicate that most NAD is synthesised to be consumed in a still poorly understood nuclear NAD metabolism, in which the non-nicotinamide (ADPR) part of the NAD molecule may have the major role.

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## Translation of oncogenic virus RNA in *Xenopus laevis* oocytes

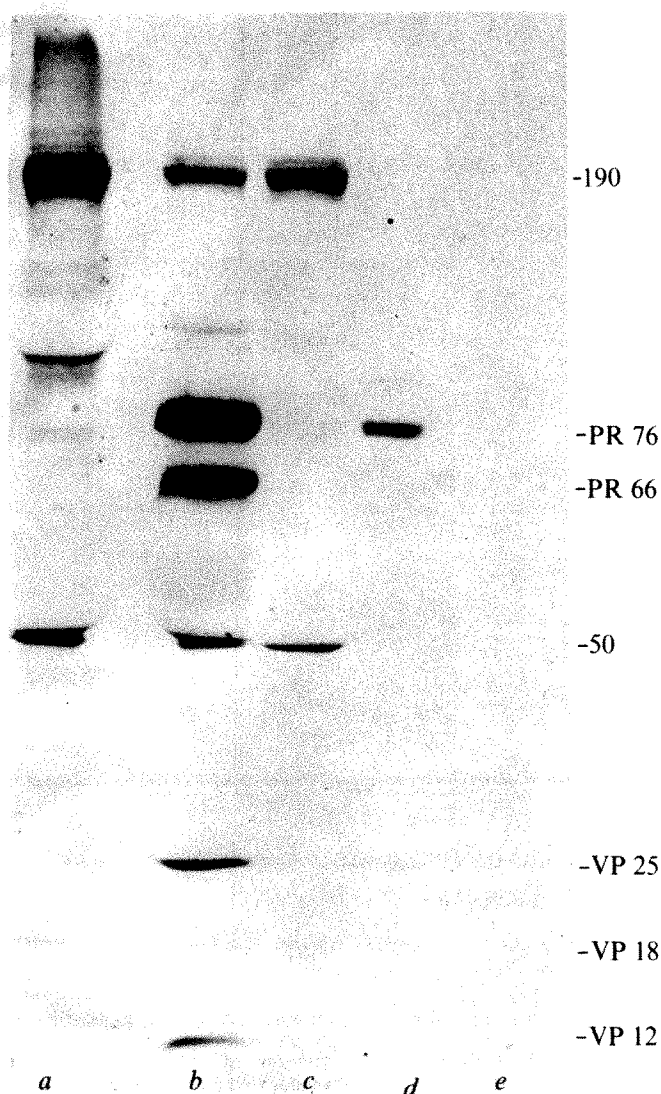
OOCYTE injection as developed by Gurdon *et al.*<sup>1-3</sup> is efficient and reliable for the assay of faithful translation of heterologous messenger RNA<sup>2-6</sup>. But, working in collaboration with Gurdon<sup>7</sup>, we found that oncogenic viral RNA was not translated to a detectable extent in oocytes. Only in a cell-free system derived from *Escherichia coli* could we translate viral RNA into distinct polypeptides in the molecular weight range of the viral group-specific proteins<sup>8</sup>. Recently we achieved translation of Rauscher leukaemia virus (RLV) RNA and avian myeloblastosis virus (AMV) RNA in various cell-free systems, using immunoprecipitation for the analysis<sup>9,10</sup>. This verified that our failure to find virus-specific polypeptides in the oocyte system programmed with oncogenic viral RNA was due to the inadequacy of the methods available to detect translation when it occurs at low efficiency. We now report the synthesis of AMV-specific precursors and structural proteins in the oocyte system, detected by specific immunoprecipitation and scintillation autoradiography.

AMV contains single stranded RNA encapsulated by several proteins and a lipid envelope. The four major proteins, the so-called group-specific (gs) antigens immunologically indistinguishable among all avian oncornaviruses<sup>11</sup>, are within the viral envelope<sup>12</sup>.

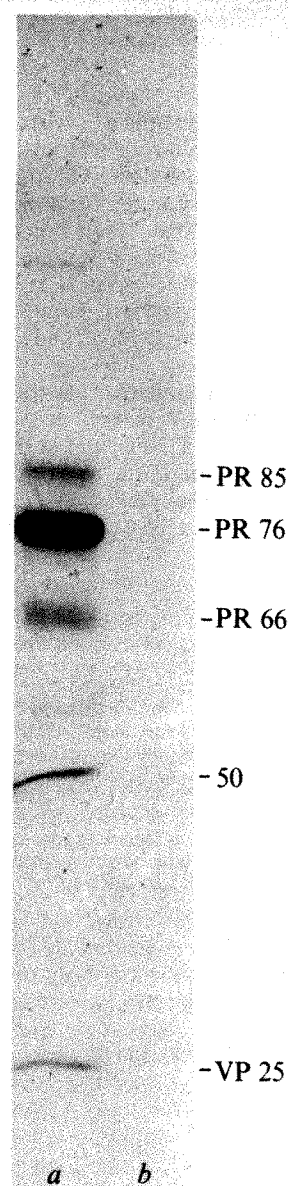
There are additional glycosylated proteins, carrying the type-specific (ts) antigens, on the surface of the virion<sup>13</sup>. It is not known how many proteins are encoded by the viral RNA and how many cellular proteins become encapsulated in the virions during budding. To determine virus-specific proteins it is important to characterise the coding capacity

of the viral RNA in conditions closely related to the *in vivo* situation.

The results of a typical translation experiment of AMV RNA are shown in Fig. 1. Because of the relatively high rate of endogenous protein synthesis of this system, immunoprecipitation with specific antisera is a prerequisite for



**Fig. 1** Autoradiography of an SDS gel electrophoresis pattern of immunoprecipitates. *Xenopus laevis* oocytes were injected by the method of message-injection described by Gurdon *et al.*<sup>1</sup> AMV was obtained from viraemic plasma<sup>18</sup> and treated with SDS and Pronase. The 60S component of AMV RNA was isolated by centrifugation in isokinetic glycerol gradients<sup>8</sup>. Isolated 60S AMV RNA was denatured by heating at 100 °C for 3 min. The AMV-specific polypeptides synthesised in the oocytes in the presence of 2 µCi <sup>35</sup>S-methionine (specific activity 478 mCi mmol<sup>-1</sup>) per oocyte were detected by indirect immunoprecipitation<sup>16</sup> with an antiserum directed against the gs antigens of AMV. Primary cultures of 1 × 10<sup>6</sup> chick embryo fibroblasts infected with AMV were pulse labelled for 30 min with 50 µCi <sup>35</sup>S-methionine (specific activity 210 Ci mmol<sup>-1</sup>) in Earle's saline. After lysis in buffer indirect immunoprecipitation was carried out to detect virus-specific polypeptides. The immunoprecipitates were analysed on sodium dodecyl sulphate (SDS)-polyacrylamide slab gel gradients (7–18%) according to Berns *et al.*<sup>17</sup>. After staining and destaining, the slab gel was treated with DMSO-PPO<sup>18</sup>, dried under vacuum and exposed in contact with a Kodak X-ray film for 3 d. *a*, Each of 20 oocytes was injected with 25 nl of sterilised distilled water; *b*, each of 10 oocytes was injected with 25 nl of native 60S AMV RNA (1 mg ml<sup>-1</sup>) in distilled water; *c*, each of 10 oocytes was injected with 25 nl of heat-denatured 35S AMV RNA (1 mg ml<sup>-1</sup>); *d*, immunoprecipitate from pulse-labelled chick embryo fibroblasts infected with AMV; *e*, immunoprecipitate from pulse-labelled uninfected chick embryo fibroblasts. PR, precursor polypeptide; VP, virion polypeptide. Numerals denote molecular weight in kdaltons.



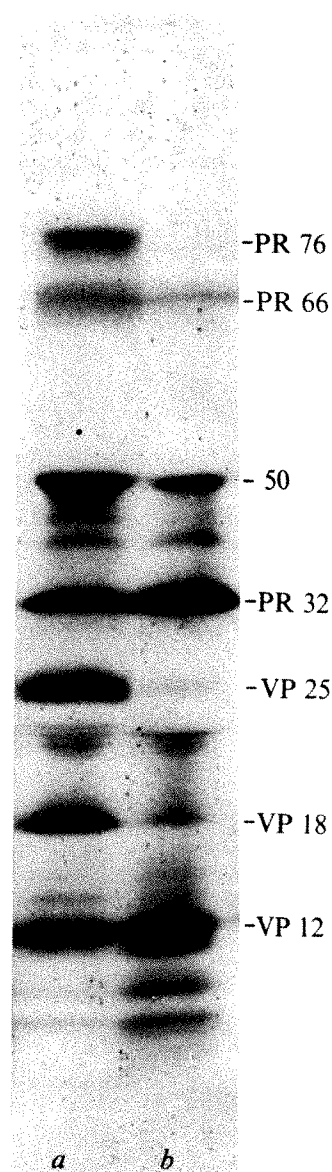
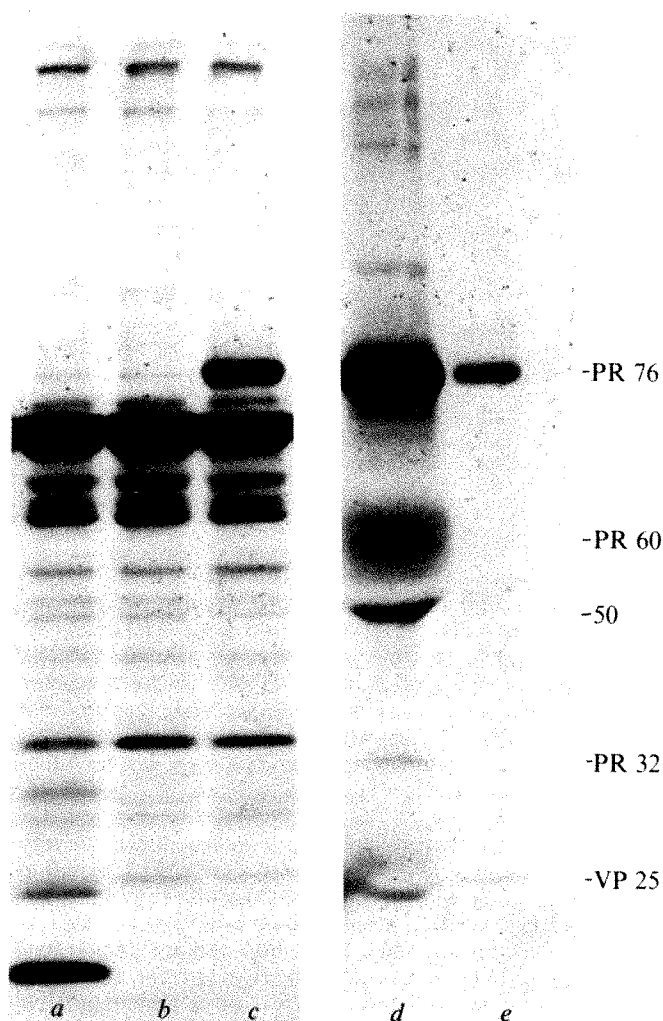
**Fig. 2** Autoradiograph of an SDS gel electrophoresis pattern of immunoprecipitates. Primary cultures of 1 × 10<sup>7</sup> chick embryo fibroblasts either uninfected or infected with AMV were pulse labelled for 30 min and immunoprecipitated as described in Fig. 1. The immunoprecipitates were analysed on slab gel gradients and exposed in contact with a Kodak X-ray film for 3 d. *a*, 1 × 10<sup>7</sup> infected cells precipitated with anti-AMV serum; *b*, 1 × 10<sup>7</sup> uninfected cells precipitated with anti-AMV serum.

detection of the viral proteins. Injection of native 60S AMV RNA results in synthesis of virus-specific polypeptides with molecular weights of 76,000, 66,000, 32,000, 25,000, 18,000 and 12,000. In contrast, denatured 35S AMV RNA leads to the synthesis of a small quantity of the precursor polypeptide with molecular weight 76,000. In chick embryo fibroblasts (CEF) infected with AMV the same precursors and structural polypeptides are found as have been synthesised after injection of oocytes with native 60S AMV RNA (compare Fig. 1*b* and *d*).

In addition to the five precursor polypeptides already described<sup>14</sup> we found in AMV-infected CEF a sixth unstable precursor of molecular weight 85,000 not present in uninfected CEF (Fig. 2). This pr85 was, however, not detectable after injection of AMV RNA into oocytes. Incubation of native 60S AMV RNA in a reticulocyte cell-free system (Fig. 3) resulted in the synthesis of the same precursors and structural polypeptides found in the oocyte system, but in

different proportions (compare Figs 1 and 3). In this cell-free system pr76 is the predominant product and almost no cleavage products are present. This probably means that the processing of AMV precursors in the oocyte and in infected CEF is almost identical, whereas in the reticulocyte cell-free system almost no cleavage occurs. In other reticulocyte preparations processing of the 76,000-dalton precursor resembled the situation *in vivo* (Fig. 4). Whereas the synthesis of the 76,000-dalton precursor polypeptide in a reticulocyte cell-free system is detectable even without immunoprecipitation (Fig. 3), as a rule immunoprecipitation procedures are necessary to visualise the small amount of virus-specific polypeptides. The specificity of the immunoprecipitation was checked as follows. Immunoprecipitation of control injections without mRNA (Fig. 1), by addition of anti-AMV serum, gives rise predominantly to two polypeptides with molecular weights of 190,000 and 50,000, respectively. The latter aspecific polypeptide is also always found when the reticulocyte lysate is the assay

**Fig. 3** Autoradiograph of the SDS gel electrophoresis pattern of products synthesised in reticulocyte cell-free incubations. Native 60S AMV RNA was incubated for 60 min at 30 °C in a reticulocyte cell-free system as described elsewhere<sup>10</sup>. The final incubation volume was 25 µl. 2 µl of the incubation mixture was analysed directly on the SDS slab gel and another 20 µl was immunoprecipitated<sup>14</sup> and analysed. The Kodak X-ray film was exposed in contact with the dried gel for 2 d. *a*, Incubation in the reticulocyte cell-free system with lens 14S mRNA; *b*, control incubation without mRNA; *c*, incubation programmed with native 60S AMV-RNA; *d*, immunoprecipitate of an incubation with native 60S AMV RNA; *e* immunoprecipitate of polypeptides present in pulse labelled AMV-infected chick embryo fibroblasts.



**Fig. 4** Autoradiography of immunoprecipitated products synthesised in the reticulocyte cell-free incubation and analysed by SDS gel electrophoresis. The analysis of the virus-specific polypeptides synthesised in the reticulocyte cell-free system were as described in the legend to Fig. 3. The X-ray film was exposed in contact with the dried gel for 3 d. *a*, Immunoprecipitation of an incubation in the reticulocyte cell-free system with native 60S AMV RNA; *b*, immunoprecipitate of an incubation in the reticulocyte lysate with denatured 35S AMV RNA.

system. The same two polypeptides are detected as the only components after injection of AMV 60S RNA into the oocyte system followed by the addition of an antiserum directed against the non-related murine RLV (not shown).

From results obtained *in vivo* and *in vitro* we conclude that AMV RNA is translated into several precursor polypeptides which are cleaved into structural viral proteins, presumably by the same relatively unspecific enzymes present in chick embryo fibroblasts, oocytes and reticulocytes. We therefore believe that the oocyte system provides a useful tool for the study of the regulation processes involved in the expression of different viral functions *in vivo*.

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*Note added in proof:* We have also succeeded in translating Rauscher leukaemia virus RNA in oocytes. The precursor of the gs antigens p65 and the processed polypeptides p30 and p15 have been detected.

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## Homology of myosin DTNB light chain with alkali light chains, troponin C and parvalbumin

THE interaction of actin and myosin which occurs during muscle contraction is regulated by changes in intracellular  $\text{Ca}^{2+}$  concentration. In vertebrates  $\text{Ca}^{2+}$  regulation is associated with troponin and tropomyosin in the thin filaments, and the myosin ATPase activity in the presence of pure actin is not  $\text{Ca}^{2+}$  sensitive. Most invertebrates possess a myosin-linked (thick filament) control system, usually in conjunction with actin-linked (thin filament) control<sup>1</sup>. Although the mechanism of thin filament regulation is fairly well characterised, that of thick filament control remains somewhat obscure (for reviews, see refs 2 and 3). This letter presents evidence for a structural and evolutionary relationship between proteins which have central roles in the two types of regulatory systems.

Myosin is composed of two heavy chains and four light chains. In rabbit white skeletal muscle, there are two structurally distinct<sup>4</sup> classes of light chains, most commonly known as alkali light chains (ALC) and DTNB light chains (DLC)<sup>5</sup>. ALCs have long been thought to have an essential function in the ATPase activity of myosin, but until recently no function could be demonstrated for DLCs. Although there is some evidence that  $\text{Ca}^{2+}$  exerts a direct effect on rabbit skeletal myosin, and that DLCs may somehow be involved in regulation of actin-myosin interaction<sup>6-11</sup>, as yet there has been no direct demonstration of myosin-linked  $\text{Ca}^{2+}$  control. DLCs, either isolated or as part of the whole myosin molecule, bind one  $\text{Ca}^{2+}$  per mol<sup>8-9,10,12</sup> but  $\text{Mg}^{2+}$  competes strongly and so significant amounts of  $\text{Ca}^{2+}$  may not be bound in physiological conditions<sup>12</sup>. In scallops (which

seem to lack troponin) specific light chains (the EDTA light chains, or ELCs) can be partially dissociated from the myosin, causing a loss of  $\text{Ca}^{2+}$  sensitivity which is restored on recombination<sup>13,14</sup>. Even though DLCs may not function as regulatory proteins in rabbit skeletal myosin, they can substitute for scallop ELC in binding and restoring  $\text{Ca}^{2+}$  sensitivity to desensitised scallop myosin<sup>15</sup>. ELCs also bind to rabbit myosin from which DLC have been partially removed, but this hybrid is not  $\text{Ca}^{2+}$  sensitive. It thus seems that both scallop and rabbit myosins contain 'regulatory' light chains, and that lack of  $\text{Ca}^{2+}$  sensitivity in rabbit myosin is due to mutations in the heavy chains<sup>3</sup>. The amino acid sequence of rabbit skeletal DLC has been determined, using standard procedures previously applied to other muscle proteins<sup>16-18</sup>; experimental details will be published elsewhere. Comparison of the DLC sequence (Fig. 1) with the partially completed sequence of ELC (R. Jakes and J. Kendrick-Jones, unpublished) further confirms that the two proteins are closely related.

Previous comparative sequence studies<sup>16,19</sup>, subsequently confirmed by more detailed analyses<sup>20-25</sup>, have suggested that the following rabbit skeletal muscle proteins are homologous (that is, they are descended from a common ancestor) and similar in three-dimensional structure: troponin C (TnC), the  $\text{Ca}^{2+}$ -binding regulatory protein of the thin filaments; ALCs, which do not bind  $\text{Ca}^{2+}$  in physiological conditions (J. Kendrick-Jones, personal communication); and  $\text{Ca}^{2+}$ -binding parvalbumin (CBP), a soluble protein of unknown function. By analogy with a carp CBP of known three-dimensional structure<sup>26</sup>, the structure of TnC seems to consist of four very similar regions (I, II, III, IV), each of which contains a 12-residue  $\text{Ca}^{2+}$ -binding segment stabilised by short  $\alpha$ -helices on either side. ALCs probably have a similar structure, although mutations in the four  $\text{Ca}^{2+}$ -binding segments have caused the loss in ability to bind in physiological conditions. The common ancestor of TnC, ALC and CBP was formed after two successive gene duplications produced a protein four times the length of an ancestral protein with a single  $\text{Ca}^{2+}$ -binding site. TnC is most closely related to the ancestor, since it has the strongest internal sequence repeats. CBP evolved by incomplete copying of the gene for the TnC-like precursor, causing deletion of region I and loss of  $\text{Ca}^{2+}$  binding in region II.

Analysis of the DLC sequence shows that it is also related to TnC. The sequences of rabbit skeletal muscle DLC, ALC (ref. 27), TnC (ref. 19) and CBP (ref. 28) are shown in Fig. 1 aligned with a uniform residue numbering system<sup>25</sup> based on the predicted structure of TnC. DLC are about equally similar to either TnC or ALC (an average of one of four residues are identical), less similar to CBP (about one of six identical residues). Of the two classes of light chains, ALC are slightly more similar to TnC. The phosphoserine-containing peptide isolated from DLC by Perrie *et al.*<sup>29</sup> is located near the N-terminus (residues NA8-NA18). As is the case with ALC, four homologous regions can be recognised in DLC, but the internal sequence repeats are very weak. Following the procedure used to predict the four  $\text{Ca}^{2+}$ -binding sites in TnC (ref. 16), the single  $\text{Ca}^{2+}$ -binding site of DLC is probably located in region I (residues abl-B3). The pattern of hydrophobic residues predicted to form the internal core of TnC is highly conserved in both DLC and ALC, suggesting that all these proteins have similar three-dimensional structures.

The finding that muscle cells contain a homologous group of structurally related proteins with seemingly diverse functions raises a number of important questions which may be answered by further comparative sequence studies. What are the structural alterations that caused the two classes of myosin light chains to evolve different functions? Since TnC and DLC are related, what further similarities exist between the two types of muscle  $\text{Ca}^{2+}$ -regulatory



	NA1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
TnC																						
DLC	X-pro	lys	lys	ala	lys	arg	arg	ala	ala	ala	Ac-asp	thr	gln	gln	ala	glu	ala	arg	SER	tyr	leu	SER
ALC											glu	gly	gly	ser	ser	asn	val	phe	SER	met	PHE	asp
	23	24	25	A1	2	3	4	5	6	7	8	9	10	11	abl	2	3	4	5	6	7	8
TnC	glu	glu	met	ILE	ALA	GLU	PHE	LYS	ala	ALA	PHE	asp	met	phe	ASP	ala	asp	GLY	gly	GLY	asp	ILE
DLC	gln	thr	GLN	ILE	gln	GLU	PHE	LYS	GLU	ALA	PHE	thr	val	ile	ASP	gln	asn	arg	asn	ser	ile	ILE
ALC	ala	asp	GLN	ILE	ALA	GLU	PHE	LYS	GLU	ALA	PHE	thr	leu	tyr	ASP	arg	thr	GLY	asn	GLY	ile	ILE
	B1	2	3	4	5	6	7	8	9	10	11	bcl	2	*	3	4	5	Cl	2	3	4	5
TnC	val	lys	glu	LEU	GLY	thr	VAL	met	ARG	met	LEU	GLY	gln	—	thr	PRO	THR	lys	GLU	GLU	LEU	ASP
DLC	lys	glu	asp	LEU	arg	ASP	thr	phe	ala	ALA	MET	GLY	arg	leu	ASN	val	lys	glu	GLU	ASP	LEU	ASP
ALC	leu	ser	gln	val	GLY	ASP	VAL	leu	ARG	ALA	LEU	GLY	thr	—	ASN	PRO	THR	asn	ala	GLU	val	LYS
CBP									Ac-ALA	MET					leu	leu						
	7	8	9	10	11	cdl	2	3	4	5	6	7			8	9	D1	2	3	4	5	6
TnC	ile	ILE	—	glu	glu	val	ASP	GLU	asp	gly	SER	GLY	thr	—	ILE	ASP	PHE	GLU	glu	PHE	LEU	val
DLC	met	—	—	asn	pro	ser	ASP	lys	gln	ala	asn	ala	lys	lys	ILE	asn	PHE	thr	val	PHE	LEU	thr
ALC	val	leu	gly	—	ala	phe	—	GLU	ala	met	gln	ser	—	—	ILE	gln	PHE	GLU	gln	PHE	thr	pro
CBP	ala	ILE	—	—	—	—	—	ala	—	—	—	—	—	—	phe	ASP	his	lys	—	—	—	—
	8	9	10	11	12	del	2	3	4	5	6	7	8	E1	2	3	4	5	6	7	8	9
TnC	MET	val	arg	gln	met	LYS	glu	asp	ala	LYS	gly	LYS	asn	GLU	GLU	glu	leu	ala	GLY	cys	PHE	ARG
DLC	MET	phe	gly	ile	—	LYS	leu	asn	LYS	asp	ala	lys	thr	GLU	GLU	val	ile	thr	GLY	ala	PHE	lys
ALC	leu	glu	ala	—	—	—	—	asn	LYS	gln	gln	lys	—	thr	GLU	ASP	phe	val	lys	val	leu	ARG
CBP	val	—	—	—	—	—	—	gly	leu	—	—	—	—	SER	thr	ASP	val	lys	lys	val	PHE	his
	11	eff	2	3	4	5	6	7	8	9	F1	2	3	4	5	6	7	8	9	10	11	
TnC	PHE	ASP	arg	asp	ala	ASP	GLY	tyr	ILE	asp	ala	GLU	GLU	LEU	ala	GLU	ILE	phe	arg	ala	ser	—
DLC	LEU	ASP	pro	GLU	GLY	lys	thr	val	ILE	lys	gly	gln	phe	LEU	glu	his	leu	LEU	thr	THR	gln	—
ALC	PHE	ASP	LYS	GLU	GLY	ASP	thr	phe	ILE	met	gln	ala	GLU	LEU	arg	phe	LEU	ala	thr	THR	leu	—
CBP	LEU	ASP	LYS	asp	lys	ser	GLY	—	—	—	—	—	GLU	LEU	gly	—	ILE	LEU	lys	gly	phe	pro
	fgl	2	3	4	5	G1	2	3	4	5	6	7	8	9	10	11	ghl	2	3	4	5	6
TnC	—	GLY	GLU	his	val	thr	asp	GLU	GLU	ILE	GLU	ser	LEU	MET	lys	asp	GLY	ASP	LYS	asn	asn	ASP
DLC	—	cys	asp	arg	phe	SER	gln	GLU	GLU	ILE	LYS	asn	met	trp	ALA	ALA	phe	pro	pro	ASP	val	GLY
ALC	—	GLY	GLU	lys	met	lys	glu	GLU	GLU	val	GLU	ala	LEU	MET	—	ALA	GLY	gln	glu	ASP	gly	GLY
CBP	asp	ala	arg	asp	leu	SER	val	lys	GLU	thr	LYS	thr	LEU	MET	ALA	ALA	GLY	ASP	LYS	ASP	ASP	GLY
	7	8	9	H1	2	3	4	5	6	7	8	9	10	11	12							
TnC	arg	ILE	ASP	phe	ASP	GLU	PHE	leu	LYS	met	met	glu	gly	val	gln-	OH						
DLC	asn	val	ASP	TYR	lys	asn	ile	cys	tyr	val	ILE	thr	his	gln-	asp	ala	lys	asp	glu	gln-	OH	
ALC	cys	ILE	asn	TYR	ala	ala	PHE	ala	LYS	his	ILE	met	ile-	gln-	OH							
CBP	lys	ILE	gly	ala	ASP	GLU	PHE	ser	thr	leu	val	ser	gln-	ser-	OH							

**Fig. 1** Alignment of the complete amino acid sequences of rabbit white skeletal muscle troponin C (TnC), myosin DTNB light chain (DLC), myosin alkali light chain (ALC) and Ca<sup>2+</sup>-binding parvalbumin (CBP). Amino acid residues are numbered in accordance with the predicted three-dimensional structure of TnC: helices are denoted A to H, from the amino to the carboxyl termini; interhelical loops are called ab, bc, and so on; residues preceding helix A are designated NA. Residues identical in two or more proteins are in capital letters, and those presumed to be involved in Ca<sup>2+</sup> binding are underlined. Asterisks indicate residues predicted to form the hydrophobic core of TnC.

systems? What differences cause TnC to bind to troponin I and troponin T in the thin filaments, whereas ALC and DLC bind to myosin heavy chains in the thick filaments? These questions bring to mind Laki's proposal<sup>30</sup>, that myosin could be considered as a complex of actin and tropomyosin (troponin had not then been discovered).

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## Erratum

In the article "Entropy production in black holes" by W. Kundt (*Nature*, **259**, 30; 1976) the second paragraph should read:

To begin with, it must be remembered that in 'normal' conditions, matter has an entropy per particle  $s \sim 1$  ( $s = SN^{-1} k^{-1}$  where  $N$  = particle number):  $s$  varies between  $\sim T/T_F \lesssim 1$  (for temperatures  $T$  below the Fermi temperature  $T_F$ ) and  $\lesssim 90$  (for dispersed hydrogen of critical cosmological density at  $\gtrsim 10^8$  K);  $s \sim 4$  for an extreme relativistic ideal gas...  $S_{bh}$  is  $10^{10} (M/M_\odot)$  times larger than  $S$  at formation.

The second  $s^a$  in line 6 and  $s^a$  in line 15 of paragraph 4 should read  $s^a$ ,  $a$ , the divergence of  $s^a$ .



# reviews

GORDON PASK has always been something of an enigma on the educational and psychological scene. Operation from a private research company, although common in the States, is still a source of puzzlement in Britain. For many years Pask remained outside any comprehensible 'establishment' with a reputation and stature that developed, and was recognised, far more outside his own country than within it. His diversity of interests, from deep epistemological problems and system theory to practical training and the construction of elaborate electromechanical automata exhibiting 'learning' or inducing training, have also made him difficult to place. His writings\* make few concessions to the reader, rarely simplifying what Pask already regards as an oversimplified view of a very complex world, and expressing it in a wide vocabulary appropriate to the richness and breadth of his conception of that world. Although one may admire an attempt to face up to a major part of the world of human acquisition of knowledge in its own terms, one may, however, also question its timeliness with regard to the intellectual and computational tools currently available to us.

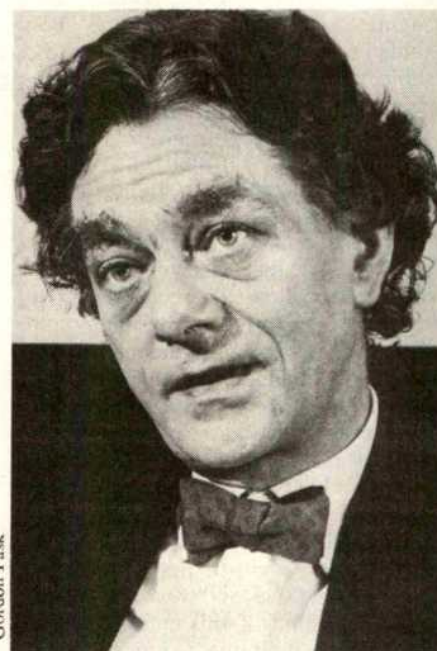
Although there are few nowadays who would question the depth or logical consistency of Pask's approach, there may be many who will question its wisdom. The history of science is full of premature attempts to consolidate major areas of knowledge, before the correct viewpoint, reasonable objectives, or appropriate logicomathematical tools have been established. Pask adopts the all-embracing methodologies of Wiener's cybernetics and Bertalanffy's general systems theory and applies them to the highest levels of human behaviour and to practical problems of teaching and training. To what extent is he successful? I cannot give a clear-cut answer to this question. Neither will I pretend that the answer lies in this one book. What is clear, however, from grappling with the material in this book, both in its original form (as published papers) and in its present integrated form, is that the questions of 'success' cannot be answered negatively.

\**Conversation, Cognition and Learning: A Cybernetic Theory and Methodology*. By Gordon Pask. Pp. xi+570. (Elsevier: Amsterdam, Oxford and New York, 1975.) Dfl.96; \$36.95.

## Common sense and the computer

**"Pask has put much effort into the development of actual teaching systems for major subject areas . . . and the practical experience and exemplary material developed is used to give life and content to the abstract, theoretical concepts put forward . . .**

**No-one who takes the trouble to immerse himself in the work can fail to gain from the experience."**



Gordon Pask

Pask has put much effort into the development of actual teaching systems for major subject areas, such as probability theory and statistics, and the practical experience and exemplary material developed is used to give life and content to the abstract, theoretical concepts put forward. There is a healthy atmosphere of operationalism throughout the work that convinces one that the cybernetic terminology is significant and applicable. No-one who takes the trouble to immerse himself in the work can fail to gain from the experience—Pask teases at basic problems from many directions at many levels and the discussion throughout is highly stimulating.

Although psychologists and system theorists will find the book of interest, its most immediate contribution is probably to studies of computer-based instruction (CAI). Pask's own use of the computer is slight—indeed, perhaps much of the insight he gives is because his structures are natural ones not distorted to fit a standard 'flow-chart' or use the keyboard of a teleprinter! His discussion of the learning and training strategies appropriate to the 'serialist' and 'holist' thinking on the one hand, and his demonstration of the complex entailment structures of comparatively simple subject areas on the other, illu-

minate major problems of CAI (and other forms of programmed instruction).

There is a paradox in our ability to develop teaching systems that work quite well for a wide range of individuals, whereas we know that there is a massive variation in student's tutorial requirements. Pask shows how performance feedback in adaptive teaching systems can tailor tuition to the individual without requiring detailed identification of his traits, provided broad differences in learning strategies can be recognised and the structure of the subject area has been thoroughly analysed. To the practising teacher such insights may seem common sense but no-one has so far successfully incorporated the common sense of the human teacher in a computer program. It is fundamental work, such as that described by Pask, that is essential if this is to become a reasonable objective.

The evaluation of Pask's work must be left to posterity—one hopes that this book will have several successors, developing and elucidating his theories and techniques. The present work must be welcomed as an opportunity to analyse, evaluate and utilise a substantial body of original and stimulating psychological and educational research.

**B. R. Gaines**



## Steroid biochemistry

*Biochemistry of Steroid Hormones.* Edited by H. J. Makin. Pp. x+358. (Blackwell Scientific: Oxford and London, 1975.) £16.50.

THE stated aims of this book are to provide a simple and concise source of information on steroid biochemistry for preclinical and BSc students, and an introduction to the subject for postgraduates. In seven chapters, several experts deal with the subject from structure and nomenclature of steroids through biosynthesis of cholesterol and steroid hormones, to control of steroidogenesis and catabolism and excretion. Two useful chapters on methods of estimation of steroids follow. The remaining five chapters deal with the physiology and pathology of steroid endocrine systems, and include chapters on the endocrinology of the menstrual cycle and pregnancy, inborn errors of corticosteroid biosynthesis and molecular mechanisms of steroid hormone action.

Generally speaking, the book fulfils its aims very well, in that it provides a comprehensive and reasonably up-to-date account of this enormous and frequently daunting subject, well illustrated by means of key experimental data, without burdening the reader with an indigestible welter of detail. Perhaps the most forbidding aspect of steroid biochemistry to a newcomer is the apparent complexity and variety of structure and interminable pathways of biosynthesis and catabolism. These problems, although not hedged, are dealt with in a sympathetic and matter-of-fact fashion. The difficulty is acknowledged and the aim is to establish and reveal the order and logic behind the structure and metabolism of these compounds. These aims are started in the first

paragraph of the first chapter, by Professor Kellie, which in itself should go a long way to allaying the fears of the reader.

As always, criticisms can be made of details, and these are inevitably subjective to some extent. For example, in Fig. 4.10, the major biosynthetic route to  $11\beta$ -hydroxy-4-androstenedione in the adrenal cortex is presented as proceeding from cortisol. Although this may be a conversion product of secreted cortisol, the major route to its biosynthesis in the adrenal gland itself is surely from C-19 precursors. Also, at the end of the book there is a pull-out scheme of steroid biosynthesis designed to assist readers of individual chapters, which may be too scanty in detail to achieve its aim.

These are, however, inevitable and minor criticisms of what is a very pleasing and useful book, which should prove of great benefit to student and teacher alike, although the price, at £16.50, might be somewhat daunting to both.

**Evan Simpson**

## Tracking down nuclear particles

*Nuclear Tracks in Solids: Principles and Applications.* By Robert L. Fleischer, P. Buford Price and Robert Walker. Pp. xix+605. (University of California: Berkeley, Los Angeles and London, November 1975.) £20.50.

FOR scientists in the UK the field of nuclear tracks in solids must surely be a frustrating example of the one that got away! Work at Harwell in the late 1950s showed that damage trails in mica produced by fission fragments could be made visible in the electron microscope. This observation was sufficient to initiate a tremendously fruit-

ful collaboration between the American authors of this book, who, within a few years, demonstrated that such damage trails could be enlarged by etching so as to be visible optically, showed that a whole range of materials other than mica would store similar tracks and applied the technique to a great variety of fields. They have now crowned their achievement with an excellent book which treats in detail many aspects of nuclear tracks in solids and conveys a vivid impression of how important this technique has become.

The book is divided into three parts. The first deals with formation of particle tracks, principles of track etching and methods by which particles may be identified by the damage trails they leave behind them. The second discusses the application of track techniques in the Earth and space sciences through fission track dating of rocks, glasses, and so on, the study of the present flux of energetic particles in space, and of stored records of past fluxes of such particles in meteorites and lunar rocks. The final part of the book deals in rather less detail with applications of the technique to nuclear physics, element mapping in rocks and other materials, radiation dosimetry and many other diverse areas in science and technology.

The book is very well written and beautifully illustrated with photographs and diagrams which considerably aid understanding of the text. The mix of information given and information only referred to in other sources is very well judged and the many references, given as appendices at the end of each chapter, have each been given a title which much increases their usefulness.

This is an excellent book worth every penny of its purchase price. It must rapidly become a standard work indispensable to those most directly involved and of great interest to many others in allied fields. **R. N. F. Walker**  
**P. H. Fowler**

## Analysing metal compounds

*Mass Spectrometry of Metal Compounds.* Edited by J. Charalambous. Pp. 297. (Butterworth: London and Boston, Massachusetts, 1975.) n.p.

THE first four chapters of the book deal with the theory of mass spectrometry, instrumentation and techniques. The next five chapters are concerned with specific groups of metal compounds, whereas the last chapter discusses analytical aspects

of the mass spectrometry of metal chelates. Finally, an appendix gives a table of nuclidic masses and abundances of the naturally occurring isotopes. There are over 400 references, many of which occur in the literature of the past decade or so, illustrating the rapid growth of inorganic mass spectrometry during the period.

There are a few minor criticisms of the book. In the first place it is surprising that in a comprehensive review of the energetics of molecular ionisation, no mention is made of the electron monochromator of Lossing, which provides the most accurate

method of determining ionisation and appearance potentials by mass spectrometry. Again the classical equation  $k(E) = v[(E - E_0)/E]^{n-1}$  is used in discussing fragmentation kinetics, in spite of the fact that it is known to be an extremely bad approximation. On the positive side, references are made to studies of negative ions, which are becoming increasingly important.

Within the confines of production from typescript, the book is well produced. It should become an essential addition to the library of anyone working in the fields covered.

**Allan Maccoll**

## Describing chemical methods

*Methodicum Chemicum: A Critical Survey of Proven Methods and their Application in Chemistry, Natural Science, and Medicine.* Edited by Friedhelm Korte. Volume 1: Analytical Methods. Part A. Purification, Wet Processes, Determination of Structure. Pp. x+1-628. Part B: Micromethods, Biological Methods, Quality Control, Automatisations. Pp. x+629-1218. (Academic: New York and London; Georg Thieme: Stuttgart, 1975.) \$98; £47.05 the two parts.

VOLUMES 1A and 1B comprise the start of an eleven-volume series which will give "a short critical description of chemical methods applied in scientific research and practice", the objective being "to provide the chemist and the scientist working in related areas of chemistry with a rapid and reliable source of information on the method applicable to his specific problem."

The subtitles of volumes 1A and 1B "Analytical Methods", is somewhat misleading, especially for 1A, as it is used in the sense of integral identification of a molecular entity and not in

that of conventional chemical analysis; moreover the emphasis is strongly biased towards organic and biological chemistry. With these reservations the editors and about 100 contributors, almost entirely European, have managed to compress into 1,200 pages a vast quantity of well presented information. The methods discussed, some of the less well known purposely given in greater detail than others, range from simple chemical operations to highly sophisticated instrumental techniques. The individual chapters provide a guidance to workers in the selection of methods appropriate to the problem being tackled and at the same time give sufficient theoretical background to enable the user to judge whether or not he can use the selected procedure himself or must employ the services of a specialist in the chosen field.

Part A deals with small-molecule separation, identification and structural analysis. Chemical and other methods are described for functional groups, but specialised techniques such as X-ray diffraction (40 pages) and various spectroscopic methods (200 pages) are dealt with in greater detail.

Part B first discusses some special physical methods covering such diverse topics as the determination of molecu-

lar weights, electron donor-acceptor complexes and electric dipole moments. Thereafter it deals with specific chemical, instrumental or biochemical techniques for the determination of both trace elements and organic constituents in a wide variety of organic samples including foods, fats, water, fuel and pharmaceuticals.

The work will provide a source of condensed information on newer techniques and, although it will not replace specialised monographs, adequate references up to 1969, sometimes up to 1972, are supplied for readers desiring more detailed information. The 53-page index, however, has omissions, no reference for example being given to the section on the use of flame photometry and atomic absorption for the analysis of fuel oils on p.955. The high cost of £48.05 will be beyond most private buyers but is justified by the amount of work that has been carried out by the authors and editors. The quality of production is excellent although somewhat marred by spelling errors and by the use of the symbol J for iodine in several places. If the following volumes maintain the quality of Volumes 1A and 1B, the series will become a reference work of major importance for all multidiscipline establishments.

R. O. Scott

## Molecular complexes

*Molecular Association.* Volume 1. Edited by R. Foster. Pp. xiv+365. (Academic: London, New York and San Francisco, August 1975.) £11.80; \$31.25.

THIS book contains four articles dealing with various aspects of electron-donor-acceptor (EDA) molecular complexes or charge-transfer (CT) complexes as they are commonly, but imprecisely, known, and with other molecular complexes. Z. G. Soos (Princeton) and D. J. Klein (Austin) give a detailed account (109pp, 340 refs) of charge transfer in solid-state complexes. This includes a phenomenological theory, a classification of  $\pi$ -molecular CT crystals, one-dimensional Hubbard models, magnetic properties of CT crystals, and the computation of model parameters. N. Kulevsky (Grand Forks) describes the dielectric properties of molecular complexes in solution, with particular reference to halogen complexes, complexes of  $\pi^*$ -acceptors, and halogenated alkane interactions (50 pp, 103 refs). K. M. C. Davis (Leicester) discusses solvent effects on 'CT complexes', including the position and intensity of the CT absorption and

fluorescence bands, charge-transfer-to-solvent (CTTS) spectra, vibrational spectra of complexes, nuclear magnetic resonance shifts, thermodynamic parameters, and complex-solvent interactions (63 pp, 334 refs). R. S. Davidson (Leicester) provides a comprehensive survey of photochemical reactions involving 'CT complexes' (120 pp, 449 refs). These are classified into reactions involving excited 'CT complexes', excimers (intermolecular and intramolecular) and exciplexes (subdivided into those with and without spectroscopic evidence).

There have been several recent books on molecular complexes, three of them written (*Organic Charge-Transfer Complexes*, Academic: London and New York, 1969) or edited (*Molecular Complexes*, 1 and 2, Elek Science, London; Crane Russak, New York, 1973 and 1974) by R. Foster, who has skilfully chosen the authors and contents of the present volume to cover topics he has not previously considered. It is doubtful whether there are sufficient gaps remaining in the literature to justify the initiation of a new series of volumes on molecular complexes under a different title selected "for bibliographical convenience." *Molecular Complexes*, Vol. 3, would have been a more accurate and convenient title for the present book. J. B. Birks

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# obituary

**Mr Ernest Gold, CB, DSO, OBE and FRS** died on January 30 at the age of 94. He was most famous for his early work on the stratosphere. In 1907 he had become the Schuster Reader in dynamic meteorology at Cambridge, and a year later he published his paper explaining the existence of the stratosphere. That a region sharply delineated by a boundary (the tropopause), where the tendency for the temperature of the air to decrease with height abruptly stops and the temperature becomes constant, seemed paradoxical. Gold's explanation of this effect, taking into account the carbon dioxide and water vapour contained in the atmosphere is still accepted, though it is a measure of the complexity of calculations in weather science that no quantitative solutions for the effect have yet been achieved. In 1910 he left Cambridge for the Met. Office, building up its stature and importance. With the advent of aviation, the need for international weather forecasting became apparent, and it was Gold's achievement to mastermind the setting up of this service, as president of the International Commission for Synoptic Weather Information, a position he held for 28 years.

**Clair L. Stong**, conductor of the 'Amateur Scientist' column in 'Scientific American' since 1957 died on Tuesday, December 9, of cancer. He was born in Douds, Iowa, and was 73 years old.

Mr. Stong, known to everyone as 'Red', first became known as an aviator. While studying electrical engineering at the University of Minnesota and the Crane Institute, he earned his keep as a stunt pilot: for a while leading his own flying circus and performing at prairie county fairs. He joined the Western Electric Co. in 1926, working in various capacities until retiring in 1962 to devote himself full-time to the Scientific American.

In his Amateur Scientist column, he opened up the 'black boxes' of modern science. He showed his readers how to build in their kitchens and garages such formidable instruments as atomic particle accelerators and cloud chambers and scintillator counters to go with them, quartz-crystal clocks, lasers, ruling engines, high-altitude rockets and both digital and analogue computers. Experiments specified in his column ranged over the frontiers of science from molecular biology (how to make amino acids) to experimental

psychology (colour vision in pigeons) to oceanography (how to make a hydrophone) to cosmology (eclipses of stars by the mountains of the moon). His readers could rely on his instructions because he had himself built nearly all the instruments, and performed nearly all the experiments he described.

His column on hang gliding in 1973 is credited with helping to make that daring sport a national pastime. An anthology of his pieces published in 1960 has been translated into a dozen languages, and with James E. Hammesfahr he wrote 'Creative Glassblowing' in 1968, a handbook for amateurs now widely consulted by laboratory technicians.

That his experiments were performed by his readers was also evident for many years from high school science fairs across the country; the American Association for the Advancement of Science at its meeting in Boston this February has scheduled a session on the role of Mr. Stong's column in stimulating careers in the sciences.

Mr. Stong will be missed by his wife, his two children, and all his readers. The Amateur Scientist column is being discontinued. **Gerard Piel**

## announcements

### International meetings

March 11, **New Particles and New Quantum Numbers**, a discussion meeting, London (The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1 5AG, UK).

April 8-10, **Natural Gas Processing and Utilisation**, Dublin (Natural Gas Conference, Institution of Chemical Engineers, P.O. Box 770, Upper Merion Street, Dublin 2, Ireland).

April 12-13, **Drug Action at the Molecular Level**, organised by the Biological Council Coordinating Committee for Symposia on Drug Action, London (Miss G. Blunt, Administrative Secretary, c/o Department of Pharmacology, University College, Gower Street, London WC1E 6BT, UK).

April 12-15, **Unsteady flow in many channels**, Newcastle-upon-Tyne (The Organising Secretary, U.F.O.C. Symposium, BHRA Fluid Engineering,

Cranfield, Bedford MK43 0AJ, UK). April 12-16, **Spring Annual Meeting**, Washington D.C. (Meetings, AGU, 1909 K Street, NW, Washington, D.C., 20006).

April 15-17, **Social and Economical Significance of Animal Production Policy of EEC and The World Strategy for Zootechnical Production**, Milan, organised by the Societa' Italiana per il Progresso della Zootechnica, Milan (Professor T. Bonadonna, Milano, Via Comelico 3, Italy).

April 20-23, **Third European Meeting on Cybernetics and Systems Research**, Vienna (Secretariat of the Austrian Society for Cybernetics Studies, Schottengasse 3, A-1010 Wien, Austria).

April 20-24, **Models and Numerical Methods Applied to the Studies of Surfaces and of Adsorbates on Surfaces**, CECAM, Paris (Professor M. Simonetta, Istituto di Chimica Fisica, University of Milan, Milan, Italy).

April 22, **European Solar Houses**,

London (The Secretary, UK-ISES, The Royal Institution, 21 Albemarle Street, London W1X 4BS, UK).

April 23-24, **Historical Biogeography, Plate Tectonics and the Changing Environment**, Corvallis, Oregon (A. J. Boucot, Department of Geology, Oregon State University, Corvallis, Oregon 97331).

April 24-30, **Radiation Protection as an Example of Action Against Modern Hazards**, Paris (Mr Gilbert Bresson, General Secretary IRPA Fourth International Congress, B.P.33,92260-Fontenay-aux-roses, France).

April 25-28, **The 22nd Annual Meeting**, Philadelphia (Institute of Environmental Sciences, Betty Peterson, Executive Director, 940 East Northwest Highway, Mount Prospect, Illinois 60056).

April 27-29, **New Materials and Processing in the Textile Industry**, Manchester (Mr J. K. Jackson, Shirley Institute, Manchester M20 8RX, UK).